Review Article

Journal of Cardiovascular Pharmacology and Therapeutics
2015, Vol. 20(3) 261-275

DOI: 10.1177/1074248414555005
cpt.sagepub.com
SAGE

How Do We Prevent the Vulnerable Atherosclerotic Plaque From Rupturing? Insights From In Vivo Assessments of Plaque, Vascular Remodeling, and Local Endothelial Shear Stress

Ioannis Andreou, MD, PhD[1], Antonios P. Antoniadis, MD, PhD[1], Koki Shishido, MD[1], Michail I. Papafaklis, MD, PhD[1], Konstantinos C. Koskinas, MD, MSc[1], Yiannis S. Chatzizisis, MD, PhD[1], Ahmet U. Coskun, PhD[1], Elazer R. Edelman, MD, PhD[1], Charles L. Feldman, ScD[1], and Peter H. Stone, MD[1]

Abstract

Coronary atherosclerosis progresses both as slow, gradual enlargement of focal plaque and also as a more dynamic process with periodic abrupt changes in plaque geometry, size, and morphology. Systemic vasculoprotective therapies such as statins, angiotensin-converting enzyme inhibitors, and antiplatelet agents are the cornerstone of prevention of plaque rupture and new adverse clinical outcomes, but such systemic therapies are insufficient to prevent the majority of new cardiac events. Invasive imaging methods have been able to identify both the anatomic features of high-risk plaque and the ongoing pathobiological stimuli responsible for progressive plaque inflammation and instability and may provide sufficient information to formulate preventive local mechanical strategies (eg, preemptive percutaneous coronary interventions) to avert cardiac events. Local endothelial shear stress (ESS) triggers vascular phenomena that synergistically exacerbate atherosclerosis toward an unstable phenotype. Specifically, low ESS augments lipid uptake and catabolism, induces plaque inflammation and oxidation, downregulates the production, upregulates the degradation of extracellular matrix, and increases cellular apoptosis ultimately leading to thin-cap fibroatheromas and/or endothelial erosions. Increases in blood thrombogenicity that result from either high or low ESS also contribute to plaque destabilization. An understanding of the actively evolving vascular phenomena, as well as the development of in vivo imaging methodologies to identify the presence and severity of the different processes, may enable early identification of a coronary plaque destined to acquire a high-risk state and allow for highly selective, focal preventive interventions to avert the adverse natural history of that particular plaque. In this review, we focus on the role of ESS in the pathobiologic processes responsible for plaque destabilization, leading either to accelerated plaque growth or to acute coronary events, and emphasize the potential to utilize in vivo risk stratification of individual coronary plaques to optimize prevention strategies to preclude new cardiac events.

Keywords

atherosclerosis, vulnerable plaque, shear stress

Introduction

Atherosclerosis research has advanced significantly over recent decades, leading to major clinical benefits. Systemic vasculoprotective medications, such as statins, angiotensin-converting enzyme inhibitors, and antiplatelet therapies, as well as lifestyle control of known risk factors such as cigarette smoking, diabetes mellitus, and elevated blood pressure, are the foundation of strategies to prevent plaque rupture. However, coronary artery disease remains a major cause of morbidity and

[1] The Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Manuscript submitted: May 15, 2014; **accepted:** August 14, 2014.

Corresponding Author:
Peter H. Stone, Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA.
Email pstone@partners.org

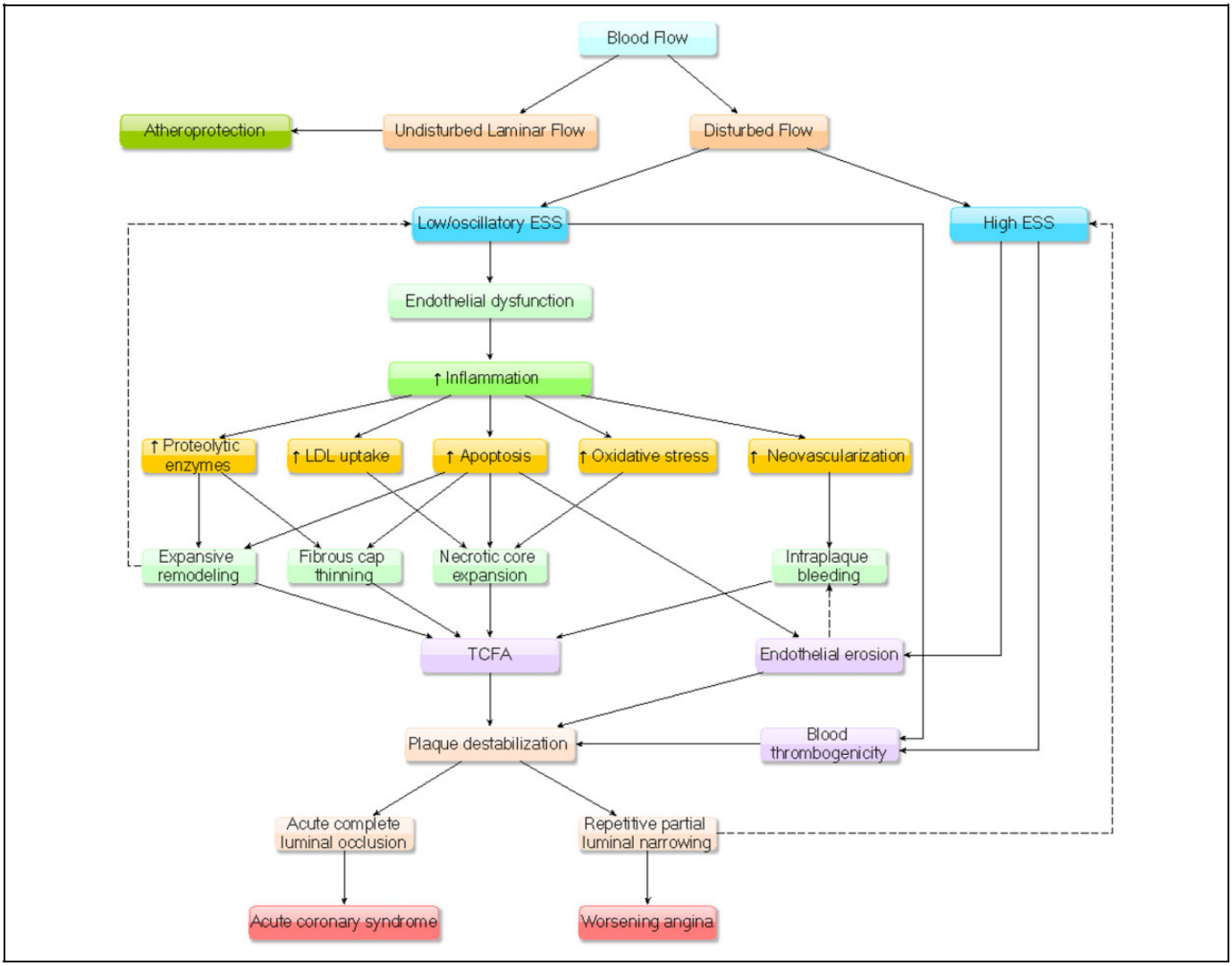

Figure 1. Endothelial shear stress (ESS)-induced plaque destabilization. Low and/or oscillatory ESS activate molecular and cellular responses in predisposed arterial areas inducing plaque buildup and thin-cap fibroatheroma (TCFA) formation, endothelial erosion, and increased blood thrombogenicity. These factors in turn are likely to cause plaque destabilization. Unstable plaques cause either abrupt complete luminal occlusion and acute coronary syndrome or accelerated plaque progression via repeated cycles of subclinical partial luminal occlusion and healing, which manifest clinically as worsening angina. Increased luminal stenosis causes high ESS, which aggravates blood-clotting tendency and may induce endothelial erosion, further contributing to plaque destabilization.

mortality. This underscores the shortcomings of current preventive, diagnostic, and therapeutic strategies and emphasizes the need for better insight into the underlying etiologic factors to enhance strategies, including local strategies, to prevent plaque rupture and thereby prevent cardiac events.

A strategy of focally directed prevention of plaque rupture is supported by the appreciation that atherosclerosis exhibits significant heterogeneity. First, the topographic localization of plaques in the coronary tree is typically asymmetrical showing a predilection for the lateral walls of bifurcations or branching points and the inner aspect of curved segments, while other areas are less frequently affected.[1] Second, the progression rate of each lesion is variable and independent, as plaques of different size and composition routinely coexist within the same patient, indeed even inside a single artery. Third, the natural history of individual lesions is diverse. Some plaques are nonobstructive, remain clinically quiescent, and may only become evident by chance during coronary imaging. Others encroach into the lumen and limit blood flow in a fixed manner presenting with stable angina. A small proportion of plaques may spontaneously activate the blood coagulation cascade and manifest as worsening ischemia or acute coronary syndromes (ACSs).[2]

Locally disturbed blood flow is a major modulator of the atherogenic process and considerably accounts for the regional, constitutional, and clinical variability of atherosclerosis (Figure 1).[3] Particularly, low endothelial shear stress (ESS) provokes molecular and cellular responses in atherosclerosis-prone sites, leading to plaque initiation and progression.[2] The local ESS microenvironment further contributes to plaque

evolution toward a stable or unstable phenotype via a multitude of mechanisms and interactions.[4] This review will focus on the role of ESS in the pathobiologic processes responsible for plaque destabilization, leading either to accelerated plaque growth or to acute coronary events. We will also discuss the clinical perspective and therapeutic implications of the in vivo ESS calculation in the early identification of high-risk lesions.

Definition and Descriptors of Plaque Destabilization

The terms vulnerable, unstable, or high-risk plaque are used interchangeably to denote an atheromatous region with propensity to trigger adverse cardiovascular events.[5] Plaque rupture or fissuring is the most common pathophysiological substrate for atherothrombosis and luminal occlusion, yet in a smaller fraction of cases, endothelial erosion may be the provoking factor and even less frequently calcified nodules overlying plaques are the culprit lesions.[6] Moreover, some incidents of plaque disruption are clinically silent because of only partial blood flow obstruction. These events of asymptomatic rupture and healing can incite rapid plaque progression and relate to untoward clinical outcomes.[7]

Although no consensus exists on all attributes of high-risk plaque, the histological hallmarks most commonly distinctive of this entity are a large lipid core (>40% of cross-sectional area), a thin fibrous cap (<65μm), and intense inflammatory cell infiltration.[8] These plaques are commonly called thin-cap fibroatheromas (TCFAs) and account for the majority of unfavorable coronary outcomes. However, a significant proportion of TCFAs may heal spontaneously during the natural course of atherosclerosis, which underscores the complexity of relating high-risk plaque to clinical events.[9] Other features suggestive of a high-risk plaque profile are endothelial denudation with superimposed platelet aggregation, disruption of the cap integrity, intraplaque hemorrhage, endothelial dysfunction, and expansive remodeling.[10-12]

The Role of Local Coronary Hemodynamics in the Development of High-Risk Plaques

Endothelial shear stress, Endothelium, and Early Atherosclerosis

Rather than a simple lining structure, the endothelial layer is an active tissue elaborating numerous molecules that determine vascular homeostasis.[13] Normal endothelium synthesizes nitric oxide (NO) from L-arginine via endothelial nitric oxide synthase (eNOS). Nitric oxide has a prominent atheroprotective role as it not only maintains vascular tone but decisively counteracts endothelial permeability, cellular proliferation, inflammation, apoptosis, and thrombosis.[14] Endothelial dysfunction is recognized as the initial critical step in the atherosclerotic process.[15] Specific receptors in the glycocalyx, a surface proteoglycan layer in endothelial cells, sense ESS (mechanosensing) and translate biomechanical forces into biochemical signals (mechanotransduction).[16] The mechanosensing process involves expression of sensors in endothelial caveolae, opening of transmembrane ion channels, activation of heterotrimeric G-proteins, and phosphorylation of the transmembrane platelet endothelial cell adhesion molecule1.[17] Endothelial shear stress subsequently activates several intra- and intercellular signaling pathways, and, via complex molecular interactions, it affects protein expression and ultimately cellular behavior within the arterial wall.[18] On the basis of different ESS values, low ESS-induced activation of nuclear factorκB (NF-κB) promotes atherogenesis, while physiologic ESS-induced transcription factor Kruppel-like factor2 mediates atheroprotection.[19]

Low and/or oscillatory ESS generates structural and functional conformational changes in the endothelial cells. Instead of being spindle shaped and aligned parallel to blood flow, endothelial cells become polygonal with an irregular and disorganized orientation.[20] Moreover, the expression of components of the endothelial glycocalyx is differentially regulated by distinct hemodynamic environments.[21] Provision of L-arginine is suppressed, and eNOS is downregulated.[22] The resulting reduced NO levels render the endothelial layer susceptible to systemic risk factors and set the stage for the initiation of atherosclerosis. Conversely, higher ESS values mediate atheroprotection via eNOS upregulation.[23] Low ESS further accentuates atherosclerosis by augmenting endothelin1 and suppressing prostacyclin production in endothelial cells.[24]

Endothelial Shear Stress, Lipid Buildup, and Plaque Progression

Lipid plaque growth with necrotic core expansion is an important element of vulnerability. Low ESS is associated with increased plaque size and lipid content. Low ESS augments cholesterol influx across the endothelial layer by increasing membrane permeability and by disrupting intercellular tight junctions via induction of endothelial apoptosis.[25,26] Additionally, low ESS triggers vascular smooth muscle cell apoptosis, which in turn exacerbates macrophage and monocyte death through multiple feedback mechanisms.[27] Death of lipid-laden foam cells in combination with ineffective clearance of the necrotic material leads to accumulation of cellular debris and plaque expansion.[28]

In computational models of coronary circulation, low ESS increases focal subendothelial low-density lipoprotein (LDL) accumulation.[29] High LDL concentration correlates well with subsequent plaque formation in these regions.[30] The increased residence time of LDL particles in this low-velocity, low-ESS coronary milieu is most likely an underlying etiologic factor predisposing to LDL penetration within the intima, which initiates, maintains, and augments atherosclerosis.

In vivo animal models readily demonstrated the role of low ESS in plaque progression as well as the synergistic effects of low ESS and hypercholesterolemia in enhanced LDL buildup within plaques leading to the formation of TCFAs.[23,31-33] In a recent study, the combination of highest cholesterol and

lowest local ESS favored the development of plaques with greatest lipid accumulation and inflammation.[34] Hence, systemic risk factors, such as hypercholesterolemia, and local risk factors, such as very low ESS, are synergistic in favoring an increasingly unstable phenotypic profile.

Local ESS and Plaque Inflammation

The intensity of local inflammation in atherosclerotic plaques determines the transition of early fibroatheromas into advanced plaques with large lipid core and thin fibrous cap and in this manner directly leads to vulnerability.[35] Low and/or oscillatory ESS contributes to recruitment of leukocytes in plaque regions by inducing synthesis of adhesion molecules (intracellular adhesion molecule1, vascular cell adhesion molecule1, and E-selectin), chemoattractant chemokines (monocyte chemoattractant protein1and interleukin8), and proinflammatory cytokines (tumor necrosis factor α and interferon-γ).[36,37] These mediators facilitate circulating leukocyte (predominantly monocyte) tethering in the endothelial membrane and diapedesis into the intima. After reaching the subendothelial layer, monocytes differentiate to macrophages, engulf oxidized LDL (oxLDL), and transform into foam cells. Foam cells produce cytokines, growth factors, reactive oxygen species, and matrix-degrading enzymes sustaining atherosclerosis progression.[38] On the other hand, physiologic ESS protects against endothelial leukocyte infiltration by reducing pseudopod projection via mechanosensing pathways.[39] Normal laminar flow antagonizes leukocyte recruitment, vascular endothelial growth factor1 induction, and E-selectin expression mediated by tumor necrosis factor α conferring atheroprotection.[40]

Animal studies assessed the in vivo effects of local low ESS in plaque progression and risk profile. Mouse carotid regions exposed to low ESS showed increased expression of inflammatory mediators and accommodated vulnerable plaques.[23,41] Furthermore, in swine models of atherosclerosis, the magnitude of low ESS related in a time- and dose-dependent manner to plaque inflammatory cell infiltration and ultimately led to the formation of TCFAs.[32,33] In the same context, local ESS plays a fundamental role in regulating the expression of receptor for advanced glycation endproducts (RAGEs), a prominent mediator of inflammation in diabetes, and inflammatory responses in the endothelium.[42] Specifically, oscillatory ESS increases RAGE expression and subsequent activation via a mechanism involving nicotinamide adenine dinucleotide phosphate oxidase-induced oxidative stress and NF-κB activation, while high ESS attenuates both RAGE expression and proinflammatory signaling via well-documented mechanisms that inhibit NF-κB.[42]

Local ESS and Oxidative Stress Within Plaque

Oxidation is critical to many aspects of the atherosclerotic process as it augments the production of oxLDL in the subendothelium, promotes inflammation, stimulates smooth muscle cell proliferation and migration to the intima, and upregulates matrix degradation, leading to advanced and destabilized plaques.[43] Endothelial shear stress locally modulates redox balance via numerous molecular interactions. Low and/or oscillating ESS exacerbate oxidation within the intima by upregulating oxidative enzymes (nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase), while suppressing antioxidant ones (superoxide dismutase and glutathione peroxidase).[44-50] Low ESS-generated reactive oxygen species in the intima react with NO to form peroxynitrite, which is an additional oxidant.[51] In addition, reactive oxygen species lead to oxidation of the eNOS cofactor tetrahydrobiopterin to dihydrobiopterin. In the absence of tetrahydrobiopterin, eNOS uncoupling occurs and superoxide formation rather than NO is induced, thus accentuating oxidation.[52] Therefore, low ESS enhances oxidation, which not only diminishes the vasculoprotective effects of NO but also further aggravates oxidative stress. Conversely, normal ESS activates the transcription factor nuclear factor erythroid 2-related factor 2 leading to increased intracellular antioxidant levels.[53] Physiologic ESS downregulates angiotensin type 1 receptors in endothelial cells, offsetting the angiotensin II-mediated oxidative stress.[54] Another potential mechanism of the antioxidant effects of physiologic ESS is an increase in the mitochondrial membrane potential, low values of which are linked to oxidative stress.[55]

Local ESS and Extracellular Matrix Turnover

Extracellular matrix (ECM) is ubiquitous in biological tissues and consists of collagen and elastin fibers interspersed with proteoglycans and glycosaminoglycans.[56] Extracellular matrix is the predominant constitutional component of both the vascular wall and the plaque fibrous cap. A dynamic equilibrium between ECM synthesis and breakdown controls the amount of ECM in the arterial wall. In plaque areas, vascular smooth muscle cells and fibroblasts produce ECM, while endothelial cells, macrophages, smooth muscle cells, T-lymphocytes, and mast cells secrete ECM-degrading enzymes, namely metalloproteinases, cathepsins, serine proteases, chymase, and tryptase.[57] In the progression of atherosclerosis, ECM degradation augments lesion formation by introducing internal elastic lamina fragmentation. This facilitates migration of vascular smooth muscle cells and macrophages to plaques and signals a shift from moderate to severe lesions.[32] In developed plaques, collagen provides biomechanical strength to the fibrous cap and safeguards its integrity. Thus, ECM degradation predisposes to instability. A porcine model of native atherosclerosis confirmed this concept by showing increased metalloproteinase and cathepsin expression levels in TCFAs.[32]

Local low ESS in vivo is associated with an enhanced expression and activity of metalloproteinases and cathepsins.[23,32] Low ESS induces proinflammatory cytokines, which in turn stimulates the release of ECM-degrading enzymes.[58] Low-ESS-induced reactive oxygen species further augment the activity of proteolytic enzymes via numerous inflammatory mediators.[59] Combined coronary flow and histopathological

studies in a swine model of native atherosclerosis showed that in coronary regions of low ESS, the internal elastic lamina undergoes local fragmentation by metalloproteinases and cathepsins. Moreover, exposure to low ESS induces the activity of ECM-catabolizing enzymes and leads to formation of TCFAs.[32] Indeed, cap thickness was inversely associated with ESS, as regions exposed to lower ESS had plaques with thinner caps.[33] Extracellular matrix degradation is particularly evident at the plaque shoulders and renders these regions more prone to disruption.[33]

In addition to contributing to ECM breakdown, local ESS variably influences ECM generation. Low ESS attenuates ECM production by smooth muscle cells by promoting their apoptosis.[60] In an animal model of carotid atherosclerosis, plaques with low ESS showed a reduced density of vascular smooth muscle cells and less collagen.[23] Recently, it was demonstrated that in regions with persistently low ESS, the combination of attenuated collagen synthesis and enhanced MMP-mediated collagen breakdown favors reduced collagen content and substantial thinning of the fibrous cap.[61] In contrast, the role of high ESS in ECM homeostasis is less well understood. It has been reported that high ESS upregulates collagen synthesis and downregulates metalloproteinase activity contributing to plaque stabilization.[62] Other studies, however, indicate that high ESS suppresses collagen synthesis and stimulates metalloproteinase activity via an inappropriately high induction of NO.[63-65] The translation of these studies and the integrated role of high ESS in the pathogenesis of plaque destabilization will be discussed in a subsequent section.

With regard to proteoglycans of the ECM, heparan sulfate has antiatherogenic properties due to reduced affinity to LDL and monocytes, as opposed to chondroitin and dermatan sulfate that are proatherogenic. Low ESS upregulates heparanase, an enzyme that destroys heparan sulfate chains in ECM and colocalizes with intense inflammatory infiltration and formation of TCFAs.[66]

Local ESS and Arterial Remodeling

Remodeling is an inherent arterial feature referring to the ability of vessels to adjust their shape in response to plaque growth or flow alterations. Generally, expansive (or positive) remodeling means that the outer arterial dimensions (ie, the external elastic membrane [EEM]) increase, while constrictive (or negative) remodeling signifies that the arterial size decreases. Under physiologic conditions, lumen size and blood flow rate act synergistically to retain ESS within normal range. In healthy arteries, high ESS stimulates expansive remodeling, while low ESS induces constrictive remodeling, in order to restore normal local flow patterns.[67] In diseased segments, however, the interactions are more complex and decisively affected by the molecular processes of atherosclerosis. Expansive remodeling is further categorized on the basis of the relation between EEM and plaque area change. Where EEM area increases equally to plaque area, the lumen is preserved (compensatory expansive remodeling). Excessive expansive remodeling occurs when EEM area increases more than plaque area, leading to lumen enlargement (overcompensation). Incomplete expansive remodeling occurs when plaque area increases more than the enlarged EEM area, leading to lumen shrinkage.

The relationship between plaque growth and vascular remodeling represents a dynamic continuum of adaptive mechanisms.[68,69] Excessive expansive remodeling is associated with indices of plaque vulnerability, unstable clinical presentation, and long-term adverse outcome.[70,71] Remodeling tightly relies upon the local dynamic ECM turnover. Low ESS, via induction of inflammation and intense ECM degradation as previously discussed, leads to TCFAs with corresponding excessive expansive remodeling.[23,32,33,72,73] This finding highlights the distinction between compensatory and excessive expansive remodeling in low-ESS regions. Although, in relatively normal arterial regions, compensatory expansive remodeling is a corrective process intended to maintain physiologic vasculoprotective ESS,[74] excessive expansive remodeling is an exaggerated response, likely related to intense local inflammation, plaque growth, and excessive wall destruction as a result of profoundly low ESS. As the plaque and arterial wall expand because of the intense local inflammation, ESS actually further decreases in these excessively enlarged regions. Under these circumstances, a vicious cycle ensues: low ESS causes intense inflammation, plaque growth, and expansive remodeling, which increases lumen size leading to perpetuation or even aggravation of low ESS and further plaque growth.[2,33]

The natural history trajectory of each lesion may involve transitions to a different remodeling pattern multiple times in its course. A multiple timepoint intravascular ultrasound (IVUS) natural history study in swine demonstrated that regions exposed to local low ESS culminated in high-risk excessive expansive remodeling.[31] In contrast, coronary segments with compensatory expansive remodeling showed higher baseline ESS values than those with excessive expansive remodeling.[31] In a recent study in a rabbit model of atherosclerosis, it was shown that low ESS, calculated with the use of in vivo magnetic resonance imaging (MRI), is associated with increased plaque burden, expansive arterial remodeling, and plaque disruption after pharmacological triggering.[75] Other studies reported that low ESS in expansively remodeled arteries is associated with increased elasticity, yet another marker of plaque instability.[76,77] A further consideration to be taken into account is that most coronary plaques are eccentric, and thus in the same cross-section, diseased areas with low ESS are contiguous with healthy segments with higher ESS. One could speculate that the excessive expansive remodeling response to plaque growth in such regions is the synergistic effect of intense ECM degradation resulting from low ESS in diseased parts and luminal dilatation in response to high ESS in the plaque-free wall.[78] A study showing that coronary segments with expansive remodeling have larger plaque-free wall areas than regions with constriction supports such a mechanism.[79]

Local ESS, Neoangiogenesis, and Intraplaque Bleeding

Intimal extension and proliferation of the vasa vasorum network is an important feature of advanced and ruptured

plaques.[80] Neovessels contribute to plaque growth by supplying inflammatory cells and mediators from the perivascular tissue. Spontaneous rupture of structurally immature and brittle vasa vasorum causes intraplaque hemorrhage.[11,81] Increased metalloproteinase activity weakens the structural scaffolding of neovessels and causes extravasation of erythrocytes and bleeding within plaques.[82] Other potential sources of intraplaque hemorrhage include subclinical plaque rupture as well as local injury of the endothelium and fibrous cap overlying the plaque.[7] Bleeding within plaques contributes in turn to plaque growth and fibrosis, possibly, by providing cholesterol from erythrocyte membranes.[83]

Low ESS induces neovascularization by causing intimal thickening and thus subintimal ischemia and by upregulating VEGF and other angiogenic stimuli.[84,85] Low ESS areas were the exclusive sites of intraplaque hemorrhage in experimental atherosclerosis. In addition, high blood pressure-induced intraplaque hemorrhage occurs in low ESS segments only.[23]

Local ESS and Endothelial Erosion

Either mechanical or functional destruction of the endothelial layer directly exposes the underlying procoagulant material, most notably von Willebrand factor and tissue factor, to circulating blood cells and plasma components. Subsequently, platelet aggregation and fibrin formation ensue. As noted previously, low and/or oscillatory ESS induce endothelial cell apoptosis, increased endothelial cell turnover, and thus likely account for a preponderance of the respective arterial regions to erode.[26,86,87] High endothelial cell turnover is postulated to relate to endothelial stem and progenitor cell senescence and exhaustion, thus reducing the vascular regenerative potential.[88] Apoptotic endothelial cells are highly proadhesive and procoagulant further favoring thrombosis.[89,90] Conversely, physiologic laminar flow contributes toward endothelial repair by enhancing stem cell proliferation and differentiation into endothelial cells.[91] In rabbit femoral arteries, oscillatory ESS led to erosive injury and endothelial detachment, leading to local thrombus formation.[92] In a porcine model of atherosclerosis, low ESS was associated with reduced endothelial coverage of the respective coronary regions.[33] Low ESS, via heparanase induction, may also damage the endothelial glycocalyx, which covers the luminal surface of the endothelium, and may provide the molecular substrate for endothelial erosion.[66,93]

The role of local high ESS on endothelial erosion is controversial. A preliminary study showed that acute exposure to high ESS leads to endothelial cell disintegration and luminal erosion.[94] This finding was corroborated in some subsequent reports,[95,96] other studies however did not confirm the abovementioned concept and showed that disturbed flow in the form of oscillatory ESS is the critical factor inducing intimal erosion in diseased arteries.[92,97] Similar inconsistency is noted with regard to the effects of high ESS in endothelial apoptosis, as some studies report that high ESS induces endothelial apoptosis,[98,99] while other studies suggest that it suppresses apoptosis.[100,101]

Plaque Biomechanics, Interactions With ESS, and the Local Role of High ESS

The dynamic interplay between ESS and plaque at sites of luminal obstruction sites largely determines plaque stability. Low ESS induces plaque growth, but such plaque affects ESS by modifying arterial geometry and altering blood flow. Endothelial shear stress increases at the throat of substantial stenoses, low ESS is more prevalent in the upstream region, while low/oscillatory ESS predominates in the downstream shoulder.[102] The plaque region downstream of minimal luminal area contains considerably more smooth muscle cells, whereas the upstream portion is more inflamed, encompassing more macrophages and more frequently shows ECM degradation and intraplaque hemorrhage.[103,104] In animal models, the downstream regions exhibited stable plaques, while the upstream segments developed vulnerable lesions.[23] Overall, it is postulated that low and/or oscillatory ESS in the downstream plaque portion induces a feedback mechanism leading to downstream plaque extension, while a high-risk plaque profile predominates in the upstream portion. Plaque rupture may ensue from ordinary hemodynamic stress in this fragile upstream area.

Since low ESS primarily mediates the vascular phenomena associated with plaque progression and vulnerability, higher ESS values are considered atheroprotective. Increases in ESS are considered the main mechanism of the beneficial impact of exercise in the cardiovascular system. Blood flow and shear rate in conduit arteries increase with exercise, and vasculoprotective molecular pathways are upregulated.[105] However, overly increased ESS also seem detrimental. Increased ESS values have been associated with plaque rupture or ulceration in some reports.[106-108] Vascular areas exposed to high flow rates may be associated with smooth muscle cell atrophy, increased macrophage infiltration, and ECM degradation.[109] High ESS pathophysiologically is linked to increased strain, which is a potential marker of instability.[110] There is evidence that exposure to high ESS sensitizes platelets so that when they subsequently reach the low ESS area they are activated at least 20-fold faster.[111] It has also been shown that platelets respond to high ESS by cellular polarization, cytoskeletal reorganization, and flow-directed migration and that even brief passage through very high ESS stenotic regions triggers platelet aggregation.[112,113] Moreover, high ESS causes platelets to secrete connective tissue growth factor, which mediates platelet adherence and to attach to von Willebrand factor leading to the formation of platelet-rich thrombus.[114] A serial human study found that high ESS is responsible for the transition of plaques toward an unstable phenotype characterized by necrotic core expansion, dense calcium accumulation, and regression of fibrous and fibrofatty tissue as assessed by radiofrequency IVUS.[115] Moreover, in a recent study, it was reported that with the progression of atherosclerotic lesions, TCFAs, determined with the use of radiofrequency IVUS on the basis of a necrotic core abutting the lumen, are less often located at low ESS regions but most frequently exposed to high ESS, which is probably the result of lumen narrowing

during plaque growth.[116] However, it is uncertain whether such a high-risk plaque profile would actually lead to clinical events.

Furthermore, it is not yet clear whether high ESS is the cause of plaque disruption or it is simply an epiphenomenon. Increased flow rate through a site of abrupt partial luminal compromise due to plaque rupture and superimposed thrombus may account for high ESS, which in this case would be the result and not the cause of rupture. From a biomechanical standpoint, the order of magnitude of ESS values is considerably smaller than that of the blood pressure-induced circumferential tensile stress in the coronary wall. Conceivably, the circumferential tensile stress is more likely to exceed the highest force that the plaque can withstand and cause rupture. In eccentric lesions, fibrous cap disruption most commonly occurs at areas exposed to the highest tensile stress as the lateral plaque shoulders.[117,118] Long-standing circumferential strain in these regions as well as substantial axial strain resulting from flow impediment is thought to introduce cap fatigue reducing tissue mechanical strength and, ultimately, causing cap fracture.[119]

Local ESS and Blood Thrombogenicity

The presence of a vulnerable plaque is not the only essential factor for adverse outcomes in the natural history of atherosclerosis. An increased responsiveness of the clotting mechanism to loss of vascular integrity is also required.[120] Low ESS promotes local blood thrombogenicity by suppressing antithrombotic and anticoagulant factors such as NO, prostacyclin, thrombomodulin, and tissue plasminogen activator.[22,121,122] Also, low ESS induces tissue factor, a strong procoagulant molecule.[123] Regional low ESS leads to increased thrombin generation[123] and increased platelet activation.[124] As already mentioned, low ESS-induced endothelial apoptosis may further contribute to an increased local thrombogenic potential.[89,90] These effects in turn stimulate a plethora of proatherogenic and plaque-destabilizing actions.[125] Interestingly, high ESS also activates platelets as discussed in the previous section. In the setting of an acute fibrous cap disruption or endothelial denudation, this ESS-mediated enhanced clotting tendency likely leads to significant fibrin formation, which gives rise to either rapid plaque progression or abrupt luminal occlusion.

Effects of Medications on Plaque Stability and Local ESS

The modification of the local ESS microenvironment and plaque stabilization may partly account for the favorable effects of established medications for coronary disease. Chronic administration of valsartan or a valsartan/simvastatin combination attenuated the proatherogenic influence of low ESS in an animal model.[126] This effect was mediated via reduced inflammation, ECM degradation, and expansive remodeling independent of their antihypertensive and hypolipidemic action.[126] Statins upregulate atheroprotective transcriptional factors and hence they are likely to counterbalance the detrimental effects of low ESS.[127] Both aspirin and ticlopidine significantly inhibit platelet aggregation under high ESS conditions.[128] In an animal model of vulnerable plaque, metoprolol treatment restored ESS values and this was associated with a reduction in inflammatory cytokines, attenuation of expansive remodeling, reduced histopathological indices of vulnerability, and a trend toward reduced plaque size and rate of rupture.[129]

In Vivo Assessment of ESS in the Detection of High-Risk Plaque and Preemptive Strategies to Prevent Plaque Rupture

As discussed previously, ESS is a critical determinant of vascular behavior and orchestrates several responses which lead to plaque destabilization. Destabilized plaques are likely to experience rapid progression of fixed, flow-impeding lesions manifesting as worsening ischemia or abrupt luminal occlusion presenting as ACS. Prevention of plaque destabilization is a major challenge in current cardiovascular medicine. Early identification of a truly high-risk lesion prone to rupture and that causes a new cardiac event is still problematic with the current diagnostic modalities. Acute coronary syndrome in a previously asymptomatic individual is a common clinical scenario, often with catastrophic consequences. In addition, residual cardiovascular morbidity exists post-ACS, despite intensive risk factor modification, pharmacological, and interventional therapy. For stable ischemia, coronary interventions currently apply only to significantly flow-limiting or occlusive lesions, overlooking potentially hazardous but nonstenotic plaques.[130]

The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study[131] was the first study to identify ostensibly high-risk plaque as associated with future coronary events and included 697 patients from the United States and Europe who underwent 3-vessel IVUS and virtual histology (VH)-IVUS assessment after percutaneous coronary intervention (PCI) for an ACS. At 1-year follow-up, there were 6.4% new major adverse cardiac events in baseline nonculprit lesions and by 3 years of follow-up there were 11.6%. The prognostic indicators at baseline for future cardiac events were large plaque burden, TCFA appearance by VH-IVUS, and minimal luminal area <40 mm^2. However, >95% of these ostensibly high-risk plaques, defined by large plaque burden or TCFA appearance, became quiescent over time and did not progress to cause a new event. The Prediction of Progression of Coronary Artery Disease and Clinical Outcomes Using Vascular Profiling of Endothelial Shear Stress and Arterial Wall Morphology (PREDICTION) study,[132] conducted in Japan, also found that new cardiac events (primarily requirement of a PCI for rapid progression of luminal obstruction), was correlated with a large plaque burden but observed as well that local low ESS was also an independent determinant of new cardiac events. Each of these 2 unique

prognostic characteristics had a positive predictive value of approximately 20% to predict new coronary events, and the combination of the 2 characteristics had a positive predictive value of approximately 40%. This study was limited by the small number of clinical events occurring in follow-up in this generally low-risk Japanese population.

Simple anatomic identification of a high-risk TCFA will not be sufficient to determine that specific plaque's likelihood to progress and cause a new cardiac event. Large plaque burden likely represents the necessary substrate of a plaque large enough to cause meaningful anatomic progression, but additional ongoing proinflammatory and proatherogenic pathobiologic stimuli that promote plaque growth and exacerbate the plaque instability and likelihood of rupture or intraplaque hemorrhage, such as low local ESS, will be necessary to optimize clinically useful prognostication of individual plaques.

Identification of coronary regions with true high-risk plaque could prompt measures to prevent adverse future sequelae utilizing intensive systemic therapy or highly selective focal interventions. Novel pharmacologic agents, as well as innovative interventional devices (ie, drug-eluting and bioresorbable stents), portend satisfactory outcomes with a low risk of adverse events.[133,134] In a smaller study, coronary segments with large plaque burden and low ESS showed greater plaque progression, while segments with pathological intimal thickening, large plaque burden, and high ESS showed increased plaque vulnerability.[135] More studies will be necessary to identify the truly high-risk plaque, focusing on the anatomic substrate responsible for plaque progression (large plaque burden) and the ongoing proinflammatory stimulus for worsening plaque vulnerability (low local ESS and/or local markers of intense inflammation or lipid-rich pool) to allow for new clinical trials to investigate preemptive revascularization interventions to prevent plaque rupture.

Other Therapeutic Modulations for Vulnerable Plaque

Apart from interventions to modify the adverse hemodynamic stimulus, other treatment options exist for vulnerable plaque, both established and emerging. Statin treatment is hitherto the cornerstone of both primary and secondary prevention pharmaceutical regimens for coronary disease. By activating series of pleiotropic pathways, statins exert potent anti-inflammatory effects, reduce circulating LDL and plaque lipid content, counteract endothelial dysfunction, and diminish blood thrombogenicity.[136] Aspirin treatment, via consistent inhibition of platelet aggregation, is an established treatment for patients after an ACS. This beneficial effect of aspirin is further potentiated by newer compounds, such as clopidogrel, prasugrel, or ticagrelor.[137] Furthermore, agents that interfere with the renin–angiotensin system have been shown to exhibit direct atheroprotective actions above and beyond their antihypertensive effect.[138] Finally, β-blockers are well established in extending life expectancy and retarding the progression of plaque in patients post-ACS.[139]

Although the above-mentioned therapies significantly reduced event rates from vulnerable plaque complications, considerable residual cardiovascular morbidity and mortality still exists to date. Therefore, the development of newer emerging agents is actively driven by clinical demand. Recombinant apolipoprotein A-1 Milano mimics the properties of nascent HDL and has been shown to induce plaque regression and association with molecular pathways pointing toward plaque stabilization.[140] Furthermore, HDL mimetic compounds enhance reverse cholesterol transport in plaque areas and hold promise toward an incremental clinical benefit in patients already receiving statin therapy.[141] Varespladib and darapladib are inhibitors of lipoprotein-associated phospholipase A2, currently in phase III of clinical trials. Despite conflicting results with respect to effects in circulating inflammatory biomarkers and invasive coronary imaging end points, darapladib has been shown to inhibit necrotic lipid core progression and therefore is potentially beneficial in plaque stabilization.[142] Newer molecular targets for vulnerable plaques have been more recently identified and their inhibition is anticipated to improve clinical outcomes. Disruption of the CD40–tumor necrosis factor receptor-associated factor 6 interaction, migration inhibitory factor receptor blocking, chemokine receptor antagonists, or adaptive immunity strategies may constitute an integral part of our therapeutic quiver in the next years.[143-146]

Adjunctive Imaging Modalities for the Detection of Vulnerable Plaque

Further to ESS assessment, the identification of plaque constituents in vivo by IVUS is capable of offering additional information to identify the high-risk plaques. Virtual histology-IVUS has shown high accuracy in the discrimination between different plaque components, thus enabling the risk stratification of plaques in low-, medium-, and high-risk categories. In the PROSPECT study, the presence of a VH-derived TCFA at baseline was an independent predictor of an ACS over a 3-year follow-up.[131] However, any potential synergistic effects of tissue characterization and the local ESS in the subsequent natural history of plaque has not yet been thoroughly investigated in large-scale clinical trials.

In the same rationale, the enhanced precision in lumen and plaque imaging by optical coherence tomography (OCT), and the incorporation of ESS analyses in OCT-derived 3-dimensional (3D) models, is expected to provide new and very useful information. Optical coherence tomography has the advantage of a considerably higher spatial resolution than IVUS enabling the characterization of vascular structure in greater detail.[147] With 3D OCT, we can investigate the association of the local hemodynamics with high-risk plaque features as the presence of severely inflamed, lipid-rich lesions with a large necrotic core and a thin fibrous cap. The 3D OCT imaging can also elucidate any etiologic contribution of coronary hemodynamics to plaque ulceration, erosion, rupture, and

thrombosis as well as to stent-positioning quality and complications after percutaneous interventions.[148,149]

Increased lipid content is a clear-cut feature of high-risk plaque, and in this setting near-infrared spectroscopy (NIRS) is a potentially useful modality. Near-infrared spectroscopy is an intravascular diagnostic methodology which is able to discover the presence of lipid-rich plaques on the basis of the absorption of near-infrared light by cholesterol. In a recent animal study, IVUS and NIRS features were able to portend the future development of high-risk plaques.[150] The subsequent development of a hybrid catheter incorporating coregistered IVUS and NIRS imaging capacity facilitates the application of this methodology in clinical settings and the decision-making process.[151]

Experience has shown that the characterization of plaque on the basis of morphological features alone is insufficient for an accurate risk assessment and prediction of future events. Molecular imaging complements traditional anatomical and structural plaque assessment by adding functional information for lesions through the use of specific probes, which in vivo identify active biological processes within the vascular wall. In experimental settings, the molecular imaging agents can identify high-risk plaque features such as intense inflammation, thrombosis, neovascularization, apoptosis, and intraplaque hemorrhage.[152] However, the translation of these initial findings in the clinical settings and especially in the coronary arteries is still under development. Fluorodeoxyglucose positron emission tomography can trace macrophage infiltration in the carotid arteries, and this is associated with future adverse events.[153]Fluorodeoxyglucose positron emission tomography is also successful in identifying inflammation in the aorta.[154] Also, in the carotid arteries, injection of ultra small super paramagnetic iron oxide particles with subsequent MRI scan was successful in identifying plaque inflammation.[155] Fluorescence-based molecular imaging utilizes near-infrared light through flexible optical fibers to detect imaging agents in the vascular wall. This approach offers high resolution and sensitivity and abolishes the use of ionizing radiation. Hybrid optical frequency domain and near-infrared fluorescence imaging enable the tracing of fingerprints of inflammation coregistered with microstructural vascular morphological assessment.[156] Indocyanine green is an FDA-approved agent for vascular imaging in ophthalmology, and it has been shown to bind lipoproteins and accumulate in inflamed tissues. Its use in in vivo atheroma near-infrared fluorescence imaging was successful in animal models.[157] With regard to noninvasive methods, coronary CT angiography has very rapidly evolved over the last years and current-generation detectors can image the entire heart in seconds and with very fine resolution. Furthermore, current advances in CT image segmentation enable us to rapidly generate 3D models of the coronary arteries for ESS assessment.[158] The most apparent benefits come from the noninvasive nature of the technique which enables its application to moderate-risk individuals, where an association of ESS with future adverse events will have implications on the primary prevention of an ACS. The application of MRI imaging for the detection of high-risk plaque remains challenging due to suboptimal spatial and temporal resolution, motion artifacts, and small target vessel size with tortuous configuration.[159] However, in experimental models, MRI-derived ESS related to increased plaque, expansive remodeling, and plaque disruption,[75] all in internal consistency with preceding studies in other species and with different modalities.

Conclusions

Investigations are ongoing to identify the truly high-risk vulnerable plaque and, consequently, to enable creation of the most appropriate prevention strategies to avert plaque rupture. At this time systemic pharmacotherapy is unlikely to be sufficient to prevent plaque rupture,[160] and highly selective local therapy, either utilizing revascularization (PCI) or local drug delivery, will likely be necessary. Invasive imaging methods can identify the anatomic appearance of high-risk vulnerable plaque, but it will be essential to characterize the factors that are responsible for an individual plaque to become more inflamed and likely to rupture, instead of evolving toward stability and quiescence.

The challenges for investigators at this time are to identify the specific constellation of high-risk features that characterize the truly highest risk plaques and screen out the vast majority of plaques and patients who are not in the highest risk category. Early identification of such high-risk plaques before they cause adverse outcomes could be a breakthrough in the management of patients with coronary artery disease as it may set the stage for highly selective prophylactic treatment strategies to prevent future coronary events, a possibility which may be both clinically invaluable and cost effective. An enormous payback for society is anticipated if these tasks are achieved, and thus these goals are worth our continued pursuit.

Author Contributions

I. Andreou contributed to conception and design, acquisition, drafted the article, critically revised the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. A. P. Antoniadis contributed to design, acquisition, drafted the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. K. Shishido, M. I. Papafaklis, K. C. Koskinas, and Y. S. Chatzizisis contributed to acquisition, drafted the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. A. U. Coskun contributed to acquisition, critically revised the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. E. R. Edelman contributed to design, critically revised the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. C. L. Feldman and P. H. Stone contributed to conception and design, critically revised the article, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received the following financial support for the research, authorship, and/or publication of this article: This work was supported by George D. Behrakis Cardiovascular Research Program.

References

1. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA*.1999;282(21):2035-2042.
2. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. 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(25):2379-2393.
3. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci*. 2000;902:230-239.
4. Koskinas KC, Chatzizisis YS, Baker AB, Edelman ER, Stone PH, Feldman CL. The role of low endothelial shear stress in the conversion of atherosclerotic lesions from stable to unstable plaque. *Curr Opin Cardiol*. 2009;24(6):580-590.
5. Ambrose JA, Srikanth S. Vulnerable plaques and patients: improving prediction of future coronary events. *Am J Med*. 2010;123(1):10-16.
6. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2000;20(5):1262-1275.
7. 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(7):934-940.
8. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol*. 2006;47(8 suppl): C13-C18.
9. Kubo T, Maehara A, Mintz GS, et al. The dynamic nature of coronary artery lesion morphology assessed by serial virtual histology intravascular ultrasound tissue characterization. *J Am Coll Cardiol*. 2010;55(15):1590-1597.
10. Fichtlscherer S, Breuer S, Zeiher AM. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation*. 2004;110(14):1926-1932.
11. Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med*. 2003;349(24):2316-2325.
12. Schoenhagen P, Ziada KM, Kapadia SR, Crowe TD, Nissen SE, Tuzcu EM. Extent and direction of arterial remodeling in stable versus unstable coronary syndromes: an intravascular ultrasound study. *Circulation*. 2000;101(6):598-603.
13. Libby P, Aikawa M, Jain MK. Vascular endothelium and atherosclerosis. *Handb Exp Pharmacol*. 2006;(176 pt 2): 285-306.
14. Li H, Forstermann U. Prevention of atherosclerosis by interference with the vascular nitric oxide system. *Curr Pharm Des*. 2009;15(27):3133-3145.
15. Giannotti G, Landmesser U. Endothelial dysfunction as an early sign of atherosclerosis. *Herz*. 2007;32(7):568-572.
16. Pahakis MY, Kosky JR, Dull RO, Tarbell JM. The role of endothelial glycocalyx components in mechanotransduction of fluid shear stress. *Biochem Biophys Res Commun*. 2007; 355(1):228-233.
17. Milovanova T, Chatterjee S, Hawkins BJ, et al. Caveolae are an essential component of the pathway for endothelial cell signaling associated with abrupt reduction of shear stress. *Biochim Biophys Acta*. 2008;1783(10):1866-1875.
18. Dhawan SS, Avati Nanjundappa RP, Branch JR, et al. Shear stress and plaque development. *Expert Rev Cardiovasc Ther*. 2010;8(4):545-556.
19. Nigro P, Abe JI, Berk BC. Flow shear stress and atherosclerosis: a matter of site specificity. *Antioxid Redox Signal*. 2011;15(5): 1405-1414.
20. Cicha I, Goppelt-Struebe M, Yilmaz A, Daniel WG, Garlichs CD. Endothelial dysfunction and monocyte recruitment in cells exposed to non-uniform shear stress. *Clin Hemorheol Microcirc*. 2008;39(1-4):113-119.
21. Koo A, Dewey CF Jr, Garcia-Cardena G. Hemodynamic shear stress characteristic of atherosclerosis-resistant regions promotes glycocalyx formation in cultured endothelial cells. *Am J Physiol Cell Physiol*. 2013;304(2):C137-C146.
22. Mun GI, Kim IS, Lee BH, Boo YC. Endothelial argininosuccinate synthetase 1 regulates nitric oxide production and monocyte adhesion under static and laminar shear stress conditions. *J Biol Chem*. 2011;286(4):2536-2542.
23. 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(23):2744-2753.
24. White SJ, Hayes EM, Lehoux S, Jeremy JY, Horrevoets AJ, Newby AC. Characterization of the differential response of endothelial cells exposed to normal and elevated laminar shear stress. *J Cell Physiol*. 2011;226(11):2841-2848.
25. Himburg HA, Grzybowski DM, Hazel AL, LaMack JA, Li XM, Friedman MH. Spatial comparison between wall shear stress measures and porcine arterial endothelial permeability. *Am J Physiol Heart Circ Physiol*. 2004;286(5):H1916-H1922.
26. Tricot O, Mallat Z, Heymes C, Belmin J, Leseche G, Tedgui A. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circulation*. 2000;101(21):2450-2453.
27. Cai Q, Lanting L, Natarajan R. Interaction of monocytes with vascular smooth muscle cells regulates monocyte survival and differentiation through distinct pathways. *Arterioscler Thromb Vasc Biol*. 2004;24(12):2263-2270.
28. Seimon T, Tabas I. Mechanisms and consequences of macrophage apoptosis in atherosclerosis. *J Lipid Res*. 2009;50 suppl: S382-S387.
29. Olgac U, Kurtcuoglu V, Poulikakos D. Computational modeling of coupled blood-wall mass transport of LDL: effects of local wall shear stress. *Am J Physiol Heart Circ Physiol*. 2008; 294(2):H909-919.
30. Olgac U, Knight J, Poulikakos D, et al. Computed high concentrations of low-density lipoprotein correlate with plaque

locations in human coronary arteries. *J Biomech*. 2011;44(13): 2466-2471.
31. Koskinas KC, Feldman CL, Chatzizisis YS, et al. Natural history of experimental coronary atherosclerosis and vascular remodeling in relation to endothelial shear stress: a serial, in vivo intravascular ultrasound study. *Circulation*. 2010;121(19): 2092-2101.
32. Chatzizisis YS, Baker AB, Sukhova GK, et al. Augmented expression and activity of extracellular matrix-degrading enzymes in regions of low endothelial shear stress colocalize with coronary atheromata with thin fibrous caps in pigs. *Circulation*. 2011;123(6):621-630.
33. Chatzizisis YS, Jonas M, Coskun AU, et al. Prediction of the localization of high-risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: an intravascular ultrasound and histopathology natural history study. *Circulation*. 2008;117(8):993-1002.
34. Koskinas KC, Chatzizisis YS, Papafaklis MI, et al. Synergistic effect of local endothelial shear stress and systemic hypercholesterolemia on coronary atherosclerotic plaque progression and composition in pigs. *Int J Cardiol*. 2013;169(6):394-401.
35. Erbel C, Dengler TJ, Wangler S, et al. Expression of IL-17A in human atherosclerotic lesions is associated with increased inflammation and plaque vulnerability. *Basic Res Cardiol*. 2011;106(1):125-134.
36. Fu Y, Hou Y, Fu C, et al. A novel mechanism of gamma/delta T-lymphocyte and endothelial activation by shear stress: the role of ecto-ATP synthase beta chain. *Circ Res*. 2011; 108(4):410-417.
37. Boisvert WA, Curtiss LK, Terkeltaub RA. Interleukin-8 and its receptor CXCR2 in atherosclerosis. *Immunol Res*. 2000;21(2-3): 129-137.
38. Zhu CH, Ying DJ, Mi JH, Zhu XH, Sun JS, Cui XP. Low shear stress regulates monocyte adhesion to oxidized lipid-induced endothelial cells via an IkappaBalpha dependent pathway. *Biorheology*. 2004;41(2):127-137.
39. Makino A, Prossnitz ER, Bunemann M, Wang JM, Yao W, Schmid-Schonbein GW. G protein-coupled receptors serve as mechanosensors for fluid shear stress in neutrophils. *Am J Physiol Cell Physiol*. 2006;290(6):C1633-C1639.
40. Chiu JJ, Lee PL, Chen CN, et al. Shear stress increases ICAM-1 and decreases VCAM-1 and E-selectin expressions induced by tumor necrosis factor-[alpha] in endothelial cells. *Arterioscler Thromb Vasc Biol*. 2004;24(1):73-79.
41. 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(3):616-626.
42. DeVerse JS, Bailey KA, Jackson KN, Passerini AG. Shear stress modulates RAGE-mediated inflammation in a model of diabetes-induced metabolic stress. *Am J Physiol Heart Circ Physiol*. 2012;302(12):H2498-H2508.
43. Hulsmans M, Holvoet P. The vicious circle between oxidative stress and inflammation in atherosclerosis. *J Cell Mol Med*. 2010;14(1-2):70-78.
44. Dimmeler S, Hermann C, Galle J, Zeiher AM. Upregulation of superoxide dismutase and nitric oxide synthase mediates the apoptosis-suppressive effects of shear stress on endothelial cells. *Arterioscler Thromb Vasc Biol*. 1999;19(3):656-664.
45. Hwang J, Ing MH, Salazar A, et al. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res*. 2003;93(12): 1225-1232.
46. 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(6):H2290-H2297.
47. Resnick N, Yahav H, Shay-Salit A, et al. Fluid shear stress and the vascular endothelium: for better and for worse. *Prog Biophys Mol Biol*. 2003;81(3):177-199.
48. 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 USA*. 2004;101(41): 14871-14876.
49. Davies PF, Civelek M. Endoplasmic reticulum stress, redox, and a proinflammatory environment in athero-susceptible endothelium in vivo at sites of complex hemodynamic shear stress. *Antioxid Redox Signal*. 2011;15(5):1427-1432.
50. Takeshita S, Inoue N, Ueyama T, Kawashima S, Yokoyama M. Shear stress enhances glutathione peroxidase expression in endothelial cells. *Biochem Biophys Res Commun*. 2000;273(1): 66-71.
51. Hsiai TK, Hwang J, Barr ML, et al. Hemodynamics influences vascular peroxynitrite formation: Implication for low-density lipoprotein apo-B-100 nitration. *Free Radic Biol Med*. 2007; 42(4):519-529.
52. Crabtree MJ, Channon KM. Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. *Nitric Oxide*. 2011;25(2):81-88.
53. Takabe W, Warabi E, Noguchi N. Anti-atherogenic effect of laminar shear stress via Nrf2 activation. *Antioxid Redox Signal*. 2011;15(5):1415-1426.
54. Ramkhelawon B, Vilar J, Rivas D, et al. Shear stress regulates angiotensin type 1 receptor expression in endothelial cells. *Circ Res*. 2009;105(9):869-875.
55. Fariss MW, Chan CB, Patel M, Van Houten B, Orrenius S. Role of mitochondria in toxic oxidative stress. *Mol Interv*. 2005;5(2): 94-111.
56. Karangelis DE, Kanakis I, Asimakopoulou AP, et al. Glycosaminoglycans as key molecules in atherosclerosis: the role of versican and hyaluronan. *Curr Med Chem*. 2010;17(33): 4018-4026.
57. Rouis M. Matrix metalloproteinases: a potential therapeutic target in atherosclerosis. *Curr Drug Targets Cardiovasc Haematol Disord*. 2005;5(6):541-548.
58. Dollery CM, Libby P. Atherosclerosis and proteinase activation. *Cardiovasc Res*. 2006;69(3):625-635.
59. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res*. 2002;90(3):251-262.
60. Qi YX, Qu MJ, Long DK, et al. Rho-GDP dissociation inhibitor alpha downregulated by low shear stress promotes vascular

smooth muscle cell migration and apoptosis: a proteomic analysis. *Cardiovasc Res*. 2008;80(1):114-122.
61. Koskinas KC, Sukhova GK, Baker AB, et al. Thin-capped atheromata with reduced collagen content in pigs develop in coronary arterial regions exposed to persistently low endothelial shear stress. *Arterioscler Thromb Vasc Biol*. 2013;33(7):1494-1504.
62. Yamane T, Mitsumata M, Yamaguchi N, et al. Laminar high shear stress up-regulates type IV collagen synthesis and down-regulates MMP-2 secretion in endothelium. A quantitative analysis. *Cell Tissue Res*. 2010;340(3):471-479.
63. Dumont O, Loufrani L, Henrion D. Key role of the NO-pathway and matrix metalloprotease-9 in high blood flow-induced remodeling of rat resistance arteries. *Arterioscler Thromb Vasc Biol*. 2007;27(2):317-324.
64. Kolpakov V, Gordon D, Kulik TJ. Nitric oxide-generating compounds inhibit total protein and collagen synthesis in cultured vascular smooth muscle cells. *Circ Res*. 1995;76(2):305-309.
65. Death AK, Nakhla S, McGrath KC, et al. Nitroglycerin upregulates matrix metalloproteinase expression by human macrophages. *J Am Coll Cardiol*. 2002;39(12):1943-1950.
66. Baker AB, Chatzizisis YS, Beigel R, et al. Regulation of heparanase expression in coronary artery disease in diabetic, hyperlipidemic swine. *Atherosclerosis*. 2010;213(2):436-442.
67. Korshunov VA, Schwartz SM, Berk BC. Vascular remodeling: hemodynamic and biochemical mechanisms underlying Glagov's phenomenon. *Arterioscler Thromb Vasc Biol*. 2007; 27(8):1722-1728.
68. 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(1):13-16.
69. Papafaklis MI, Koskinas KC, Chatzizisis YS, Stone PH, Feldman CL. In-vivo assessment of the natural history of coronary atherosclerosis: vascular remodeling and endothelial shear stress determine the complexity of atherosclerotic disease progression. *Curr Opin Cardiol*. 2010;25(6):627-638.
70. Kroner ES, van Velzen JE, Boogers MJ, et al. Positive remodeling on coronary computed tomography as a marker for plaque vulnerability on virtual histology intravascular ultrasound. *Am J Cardiol*. 2011;107(12):1725-1729.
71. Okura H, Kobayashi Y, Sumitsuji S, et al. Effect of culprit-lesion remodeling versus plaque rupture on three-year outcome in patients with acute coronary syndrome. *Am J Cardiol*. 2009; 103(6):791-795.
72. 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(4):438-444.
73. Stone PH, Coskun AU, Kinlay S, et al. Regions of low endothelial shear stress are the sites where coronary plaque progresses and vascular remodelling occurs in humans: an in vivo serial study. *Eur Heart J*. 2007;28(6):705-710.
74. Carter HH, Dawson EA, Birk GK, et al. Effect of SR manipulation on conduit artery dilation in humans. *Hypertension*. 2013; 61(1):143-150.
75. Phinikaridou A, Hua N, Pham T, Hamilton JA. Regions of low endothelial shear stress colocalize with positive vascular remodeling and atherosclerotic plaque disruption: an in vivo magnetic resonance imaging study. *Circ Cardiovasc Imaging*. 2013;6(2): 302-310.
76. Duivenvoorden R, Vanbavel E, de Groot E, et al. Endothelial shear stress: a critical determinant of arterial remodeling and arterial stiffness in humans—a carotid 3.0-T MRI study. *Circ Cardiovasc Imaging*. 2010;3(5):578-585.
77. Baldewsing RA, Schaar JA, Mastik F, van der Steen AF. Local elasticity imaging of vulnerable atherosclerotic coronary plaques. *Adv Cardiol*. 2007;44:35-61.
78. 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(8):401-407.
79. 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(1):17-23.
80. 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(14):2032-2038.
81. Sluimer JC, Kolodgie FD, Bijnens AP, et al. Thin-walled microvessels in human coronary atherosclerotic plaques show incomplete endothelial junctions relevance of compromised structural integrity for intraplaque microvascular leakage. *J Am Coll Cardiol*. 2009;53(17):1517-1527.
82. de Nooijer R, Verkleij CJN, von der Thüsen JH, et al. Lesional overexpression of matrix metalloproteinase-9 promotes intraplaque hemorrhage in advanced lesions but not at earlier stages of atherogenesis. *Arterioscler Thromb Vasc Biol*. 2006;26(2): 340-346.
83. Giannoglou GD, Koskinas KC, Tziakas DN, et al. Total cholesterol content of erythrocyte membranes and coronary atherosclerosis: an intravascular ultrasound pilot study. *Angiology*. 2009;60(6):676-682.
84. Hohberg M, Knochel J, Hoffmann CJ, et al. Expression of ADAMTS1 in endothelial cells is induced by shear stress and suppressed in sprouting capillaries. *J Cell Physiol*. 2011; 226(2):350-361.
85. Chu TJ, Peters DG. Serial analysis of the vascular endothelial transcriptome under static and shear stress conditions. *Physiol Genomics*. 2008;34(2):185-192.
86. Xu Q. Disturbed flow-enhanced endothelial turnover in atherosclerosis. *Trends Cardiovasc Med*. 2009;19(6):191-195.
87. Vasa M, Breitschopf K, Zeiher AM, Dimmeler S. Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ Res*. 2000;87(7):540-542.
88. Kovacic JC, Moreno P, Hachinski V, Nabel EG, Fuster V. Cellular senescence, vascular disease, and aging: part 1 of a 2-part review. *Circulation*. 2011;123(15):1650-1660.
89. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood*. 1999;93(11):3831-3838.

90. Potapova IA, Cohen IS, Doronin SV. Apoptotic endothelial cells demonstrate increased adhesiveness for human mesenchymal stem cells. *J Cell Physiol*. 2009;219(1):23-30.
91. Zhou B, Margariti A, Zeng L, Xu Q. Role of histone deacetylases in vascular cell homeostasis and arteriosclerosis. *Cardiovasc Res*. 2011;90(3):413-420.
92. Sumi T, Yamashita A, Matsuda S, et al. Disturbed blood flow induces erosive injury to smooth muscle cell-rich neointima and promotes thrombus formation in rabbit femoral arteries. *J Thromb Haemost*. 2010;8(6):1394-1402.
93. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch*. 2007;454(3):345-359.
94. Fry DL. Acute vascular endothelial changes associated with increased blood velocity gradients. *Circ Res*. 1968;22(2): 165-197.
95. Bernardo A, Ball C, Nolasco L, Choi H, Moake JL, Dong JF. Platelets adhered to endothelial cell-bound ultra-large von Willebrand factor strings support leukocyte tethering and rolling under high shear stress. *J Thromb Haemost*. 2005;3(3):562-570.
96. Wechezak AR, Coan DE, Viggers RF, Sauvage LR. Dextran increases survival of subconfluent endothelial cells exposed to shear stress. *Am J Physiol*. 1993;264(2 pt 2):H520-H525.
97. Langille LB. Integrity of arterial endothelium following acute exposure to high shear stress. *Biorheology*. 1984;21(3):333-346.
98. Dolan JM, Meng H, Singh S, Paluch R, Kolega J. High fluid shear stress and spatial shear stress gradients affect endothelial proliferation, survival, and alignment. *Ann Biomed Eng*. 2011; 39(6):1620-1631.
99. Macario DK, Entersz I, Abboud JP, Nackman GB. Inhibition of apoptosis prevents shear-induced detachment of endothelial cells. *J Surg Res*. 2008;147(2):282-289.
100. Metaxa E, Meng H, Kaluvala SR, Szymanski MP, Paluch RA, Kolega J. Nitric oxide-dependent stimulation of endothelial cell proliferation by sustained high flow. *Am J Physiol Heart Circ Physiol*. 2008;295(2):H736-H742.
101. Zeng Y, Qiao Y, Zhang Y, Liu X, Wang Y, Hu J. Effects of fluid shear stress on apoptosis of cultured human umbilical vein endothelial cells induced by LPS. *Cell Biol Int*. 2005;29(11): 932-935.
102. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat Clin Pract Cardiovasc Med*. 2009;6(1):16-26.
103. Segers D, Helderman F, Cheng C, et al. Gelatinolytic activity in atherosclerotic plaques is highly localized and is associated with both macrophages and smooth muscle cells in vivo. *Circulation*. 2007;115(5):609-616.
104. Fagerberg B, Ryndel M, Kjelldahl J, et al. Differences in lesion severity and cellular composition between in vivo assessed upstream and downstream sides of human symptomatic carotid atherosclerotic plaques. *J Vasc Res*. 2010;47(3):221-230.
105. Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ. Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci Sports Exerc*. 2009;41(5):1072-1079.
106. Fukumoto Y, Hiro T, Fujii T, et al. Localized elevation of shear stress is related to coronary plaque rupture: a 3-dimensional intravascular ultrasound study with in-vivo color mapping of shear stress distribution. *J Am Coll Cardiol*. 2008;51(6):645-650.
107. Groen HC, Gijsen FJ, van der Lugt A, et al. Plaque rupture in the carotid artery is localized at the high shear stress region: a case report. *Stroke*. 2007;38(8):2379-2381.
108. Tang D, Teng Z, Canton G, et al. Sites of rupture in human atherosclerotic carotid plaques are associated with high structural stresses: an in vivo MRI-based 3D fluid–structure interaction study. *Stroke*. 2009;40(10):3258-3263.
109. Kenagy RD, Fischer JW, Davies MG, et al. Increased plasmin and serine proteinase activity during flow-induced intimal atrophy in baboon PTFE grafts. *Arterioscler Thromb Vasc Biol*. 2002;22(3):400-404.
110. Gijsen FJ, Mastik F, Schaar JA, et al. High shear stress induces a strain increase in human coronary plaques over a 6-month period. *EuroIntervention*. 2011;7(1):121-127.
111. Sheriff J, Bluestein D, Girdhar G, Jesty J. High-shear stress sensitizes platelets to subsequent low-shear conditions. *Ann Biomed Eng*. 2010;38(4):1442-1450.
112. Kraemer BF, Schmidt C, Urban B, et al. High shear flow induces migration of adherent human platelets. *Platelets*. 2011;22(6): 415-421.
113. Para A, Bark D, Lin A, Ku D. Rapid platelet accumulation leading to thrombotic occlusion. *Ann Biomed Eng*. 2011;39(7): 1961-1971.
114. Reininger AJ, Heijnen HF, Schumann H, Specht HM, Schramm W, Ruggeri ZM. Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. *Blood*. 2006;107(9):3537-3545.
115. Samady H, Eshtehardi P, McDaniel MC, et al. 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(7):779-788.
116. Wentzel JJ, Schuurbiers JC, Gonzalo Lopez N, et al. In vivo assessment of the relationship between shear stress and necrotic core in early and advanced coronary artery disease. *EuroIntervention*. 2013;9(8):989-995; discussion 995.
117. Richardson PD, Davies MJ, Born GV. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet*. 1989;2(8669):941-944.
118. Kumar RK, Balakrishnan KR. Influence of lumen shape and vessel geometry on plaque stresses: possible role in the increased vulnerability of a remodelled vessel and the ''shoulder'' of a plaque. *Heart*. 2005;91(11):1459-1465.
119. Doriot PA. Estimation of the supplementary axial wall stress generated at peak flow by an arterial stenosis. *Phys Med Biol*. 2003;48(1):127-138.
120. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. *Circulation*. 2003;108(15):1664-1672.
121. Di Francesco L, Totani L, Dovizio M, et al. Induction of prostacyclin by steady laminar shear stress suppresses tumor

necrosis factor-alpha biosynthesis via heme oxygenase-1 in human endothelial cells. *Circ Res*. 2009;104(4):506-513.
122. Lin Z, Kumar A, SenBanerjee S, et al. Kruppel-like factor 2 (KLF2) regulates endothelial thrombotic function. *Circ Res*. 2005;96(5):e48-e57.
123. Yin W, Shanmugavelayudam SK, Rubenstein DA. The effect of physiologically relevant dynamic shear stress on platelet and endothelial cell activation. *Thromb Res*. 2011;127(3):235-241.
124. Rubenstein DA, Yin W. Quantifying the effects of shear stress and shear exposure duration regulation on flow induced platelet activation and aggregation. *J Thromb Thrombolysis*. 2010;30(1): 36-45.
125. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med*. 2011;364(18): 1746-1760.
126. Chatzizisis YS, Jonas M, Beigel R, et al. Attenuation of inflammation and expansive remodeling by valsartan alone or in combination with simvastatin in high-risk coronary atherosclerotic plaques. *Atherosclerosis*. 2009;203(2):387-394.
127. Takahashi S, Papafaklis MI, Sakamoto S, et al. The effect of statins on high-risk atherosclerotic plaque associated with low endothelial shear stress. *Curr Opin Lipidol*. 2011;22(5): 358-364.
128. Matsumoto M, Kawaguchi S, Ishizashi H, et al. Platelets treated with ticlopidine are less reactive to unusually large von Willebrand factor multimers than are those treated with aspirin under high shear stress. *Pathophysiol Haemost Thromb*. 2005;34(1): 35-40.
129. Liang C, Xiaonan L, Xiaojun C, et al. Effect of metoprolol on vulnerable plaque in rabbits by changing shear stress around plaque and reducing inflammation. *Eur J Pharmacol*. 2009; 613(1-3):79-85.
130. Kushner FG, Hand M, Smith SC Jr, et al. 2009 focused updates: ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction (updating the 2004 guideline and 2007 focused update) and ACC/AHA/SCAI guidelines on percutaneous coronary intervention (updating the 2005 guideline and 2007 focused update) a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2009;54(23): 2205-2241.
131. Stone GW, Maehara A, Lansky AJ, et al. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011; 364(3):226-235.
132. 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(2):172-181.
133. Silber S, Gutierrez-Chico JL, Behrens S, et al. Effect of paclitaxel elution from reservoirs with bioabsorbable polymer compared to a bare metal stent for the elective percutaneous treatment of de novo coronary stenosis: the EUROSTAR-II randomised clinical trial. *EuroIntervention*. 2011;7(1):64-73.
134. Reifart N, Hauptmann KE, Rabe A, Enayat D, Giokoglu K. Short and long term comparison (24 months) of an alternative sirolimus-coated stent with bioabsorbable polymer and a bare metal stent of similar design in chronic coronary occlusions: the CORACTO trial. *EuroIntervention*. 2010;6(3):356-360.
135. Corban MT, Eshtehardi P, Suo J, et al. Combination of plaque burden, wall shear stress, and plaque phenotype has incremental value for prediction of coronary atherosclerotic plaque progression and vulnerability. *Atherosclerosis*. 2014;232(2):271-276.
136. Mihos CG, Pineda AM, Santana O. Cardiovascular effects of statins, beyond lipid-lowering properties. *Pharmacol Res*. 2014; 88C:12-19.
137. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361(11):1045-1057.
138. Mann JFE, Schmieder RE, McQueen M, et al. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *Lancet*. 2008;372(9638):547-553.
139. Sipahi I, Tuzcu EM, Wolski KE, et al. Beta-blockers and progression of coronary atherosclerosis: pooled analysis of 4 intravascular ultrasonography trials. *Ann Intern Med*. 2007;147(1): 10-18.
140. Giannarelli C, Cimmino G, Ibanez B, et al. Acute ApoA-I Milano administration induces plaque regression and stabilisation in the long term. *Thromb Haemost*. 2012;108(6):1246-1248.
141. Joy TR. Novel HDL-based therapeutic agents. *Pharmacol Ther*. 2012;135:18-30.
142. Serruys PW, Garcia-Garcia HM, Buszman P, et al. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation*. 2008;118(11):1172-1182.
143. Lutgens E, Lievens D, Beckers L, et al. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. *J Exp Med*. 2010;207(2):391-404.
144. Koenen RR, Weber C. Therapeutic targeting of chemokine interactions in atherosclerosis. *Nat Rev Drug Discov*. 2010;9(2): 141-153.
145. Klingenberg R, Lebens M, Hermansson A, et al. Intranasal immunization with an apolipoprotein B-100 fusion protein induces antigen-specific regulatory T cells and reduces atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2010;30(5):946-952.
146. Kraemer S, Lue H, Zernecke A, et al. MIF-chemokine receptor interactions in atherogenesis are dependent on an N-loop-based 2-site binding mechanism. *FASEB J*. 2011;25(3):894-906.
147. Tian J, Ren X, Vergallo R, et al. Distinct morphological features of ruptured culprit plaque for acute coronary events compared to those with silent rupture and thin-cap fibroatheroma: a combined optical coherence tomography and intravascular ultrasound study. *J Am Coll Cardiol*. 2014;63(21):2209-2216.
148. Okamura T, Onuma Y, Yamada J, et al. 3D optical coherence tomography: new insights into the process of optimal rewiring of side branches during bifurcational stenting. [published online February 17, 2014] *EuroIntervention*. 2014.
149. Papafaklis MI, Bourantas CV, Yonetsu T, et al. Anatomically correct three-dimensional coronary artery reconstruction using frequency domain optical coherence tomographic and

angiographic data: head-to-head comparison with intravascular ultrasound for endothelial shear stress assessment in humans [published online June 30, 2014]. *EuroIntervention*. 2014.

150. Patel D, Hamamdzic D, Llano R, et al. Subsequent development of fibroatheromas with inflamed fibrous caps can be predicted by intracoronary near infrared spectroscopy. *Arterioscler Thromb Vasc Biol*. 2013;33(2):347-353.
151. Garg S, Serruys PW, van der Ent M, et al. First use in patients of a combined near infra-red spectroscopy and intra-vascular ultrasound catheter to identify composition and structure of coronary plaque. *EuroIntervention*. 2009;5(6):755-756.
152. Kusters DH, Tegtmeier J, Schurgers LJ, Reutelingsperger CP. Molecular imaging to identify the vulnerable plaque—from basic research to clinical practice. *Mol Imaging Biol*. 2012; 14(5):523-533.
153. Marnane M, Merwick A, Sheehan OC, et al. Carotid plaque inflammation on 18F-fluorodeoxyglucose positron emission tomography predicts early stroke recurrence. *Ann Neurol*. 2012;71(5):709-718.
154. Vijayakumar J, Subramanian S, Singh P, et al. Arterial inflammation in bronchial asthma. *J Nucl Cardiol*. 2013;20(3):385-395.
155. Trivedi RA, Mallawarachi C, JM UK-I, et al. Identifying inflamed carotid plaques using in vivo USPIO-enhanced MR imaging to label plaque macrophages. *Arterioscler Thromb Vasc Biol*. 2006;26(7):1601-1606.
156. Jaffer FA, Calfon MA, Rosenthal A, et al. Two-dimensional intravascular near-infrared fluorescence molecular imaging of inflammation in atherosclerosis and stent-induced vascular injury. *J Am Coll Cardiol*. 2011;57(25):2516-2526.
157. Vinegoni C, Botnaru I, Aikawa E, et al. Indocyanine green enables near-infrared fluorescence imaging of lipid-rich, inflamed atherosclerotic plaques. *Sci Transl Med*. 2011;3(84): 84ra45.
158. Rybicki FJ, Melchionna S, Mitsouras D, et al. Prediction of coronary artery plaque progression and potential rupture from 320-detector row prospectively ECG-gated single heart beat CT angiography: Lattice Boltzmann evaluation of endothelial shear stress. *Int J Cardiovasc Imaging*. 2009;25(2 suppl): 289-299.
159. Nappi C, Acampa W, Pellegrino T, Petretta M, Cuocolo A. Beyond ultrasound: advances in multimodality cardiac imaging [published online July 19, 2014]. *Intern Emerg Med*. 2014.
160. Cannon CP, Braunwald E, McCabe CH, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*. 2004;350(15):1495-1504.