Dialogues in Cardiovascular Medicine

Aim & Scope

Dialogues in Cardiovascular Medicine is a peer-reviewed, quarterly journal for cardiologists and physicians with an interest in cardiology. In order to promote in-depth understanding of the current body of knowledge on a specific area of cardiovascular medicine, each issue aims to provide a comprehensive analysis of a single topic. The lead article explores each topic in concise detail. Three pressing questions that dominate the field are identified and given personal replies by undisputed authorities in the expert answers section. The summaries of ten seminal papers put the topic into historical perspective. The fascinomata cardiologica section takes a thought-provoking and at times unconventional approach to cardiology from a variety of vantage points. Finally, a selected bibliography of one hundred key papers is available for those readers who wish to undertake a more exhaustive investigation of the topic. Dialogues offers unique coverage of the state of the art in clinical cardiology. The journal is indexed in medical databases and is part of the continuing medical education programs of several major international cardiological societies.

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The Vulnerable Plaque

Editorial
The vulnerable plaque: dealing with a looming landslide - R. Ferrari, K. Fox, F. Crea, G. Liuzzo 3

Lead Article
The vulnerable plaque in the natural history of atherosclerosis - F. Crea, G. Liuzzo 5

Expert Answers to Three Key Questions
How can the vulnerable plaque be identified? - C. M. Campos, C. V. Bourantas, H. M. Garcia-Garcia, P. A. Lemos, P. W. Serruys 29

What are the invasive approaches to the vulnerable plaque? - B. Meier 36

What are the pharmacological approaches to treat the vulnerable plaque? - K. Fox 41

Summaries of Ten Seminal Papers - F. Crea 49

The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina – G. Liuzzo and others
Plaque rupture and sudden death related to exertion in men with coronary artery disease – A. P. Burke and others
Perturbation of the T cell repertoire in patients with unstable angina – G. Liuzzo and others
Widespread coronary inflammation in unstable angina A. Baffon and others
Is there a vulnerable plaque? – A Maseri and V. Fuster

Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes G. S. Abela and others
High levels of systemic myeloperoxidase are associated with coronary plaque erosion in patients with acute coronary syndromes: a clinicopathological study – G. Ferrante and others
Enhanced Rho-kinase activity in circulating neutrophils of patients with vasospastic angina: a possible biomarker for diagnosis and disease activity assessment – Y. Kikuchi and others
Imaging intraplaque inflammation in carotid atherosclerosis with 11C-PK11195 positron emission tomography/computed tomography – O. Gaemperli and others
Immune effector mechanisms implicated in atherosclerosis: from mice to humans – P. Libby and others

Bibliography of One Hundred Key Papers 61
Without any doubt, the marked decline in cardiovascular mortality experienced in the majority of the Western world represents one of the true success stories of modern medicine. This success may be ascribed both to the treatment of acute coronary syndromes (ACS) and chronic coronary artery disease (CAD), as well as to risk factor modification. Nonetheless, despite undeniable advances, the coronary epidemic rages on—the words are not too strong. One person dies every minute in the USA from a coronary event, and CAD accounts for between 16% and 25% of all deaths in European men. According to current estimates, in 2020, CAD will be responsible for 11.1 million deaths throughout the world, thus remaining for years, maybe decades to come, the leading cause of death.

Since the first lucid description of CAD (angina) in early 1772 by William Heberden, great strides have been made in the understanding of the processes that result in the development and progression of atherosclerosis. Fatty streaks have been observed in human fetal arteries and develop at increasingly earlier ages as today’s children become less and less physically active. The plaque stage starts appearing at puberty in boys, much later in girls. Hence atherosclerosis strikes early, and is truly a lifelong disease, a lifelong threat.

As to the mechanism, coronary plaque thrombosis is present in the majority of patients that die a cardiovascular death. The lesion substrate prone to thrombosis is termed “vulnerable plaque,” and this is the topic of this issue of Dialogues in Cardiovascular Medicine—a topic already discussed in several issues of the journal in the past. The pace of change is such in this field that it calls for being revisited at regular intervals.

The outcome of this brittle, unstable, fissuring, eroding—in other words highly vulnerable—plaque is the impending doom of rupture, a catastrophe not unlike a mountain landslide or an avalanche as in the cartoon pictured here (see on next page). This “landslide” is responsible for 75% of acute coronary syndromes.
So the cartoon indeed poses the essential question: what can we do?

Lead authors Filippo Crea and Giovanna Liuzzo give a clear insight into the factors that allow atherosclerosis to progress from the benign phenotype characterized by intimal thickening to the more dangerous fibroatheroma, characterized by a necrotic core. As the plaque becomes inflamed and/or metabolic processes activate the fibrous cap, it becomes thin and prone to rupture or to erosion. Regardless, the precipitating clinical event is always coronary thrombosis.

Moving from pathophysiology to the patient, a more clinically relevant definition of a vulnerable plaque is a lesion that places patients at risk for future major adverse cardiac events (MACE). It follows that the identification of those plaques at risk before they become symptomatic would allow stratification of the risk and facilitate coronary thrombosis prevention. Numerous candidates for invasive and noninvasive imaging modalities have been proposed such as multidetector computerized tomography, radiofrequency intravascular ultrasound (RF-IVUS), optical coherence tomography (OCT), and near-infrared spectroscopy (NIRS). Patrick W. Serruys and his team from Rotterdam (Christos V. Bourantas, Hector M. Garcia-Garcia, and Pedro A. Lemos) take us through the advantages and disadvantages of all of the different modalities. The understanding of the pathophysiological processes and the ability to detect vulnerable plaques has significant implications for future research and treatment. Bernhard Meier reviews the various invasive approaches for assessment (still too costly and risky, and at the end of the day of little predictive value) and care of the vulnerable plaque (balloon angioplasty [POBA], bare-metal and drug-eluting stents). Keith Fox goes through the pharmacological approaches—those that aim to modify risk factors systemically, newer therapies targeted at the plaque itself to reduce the risk of rupture and complications, involving such cutting-edge technologies as microspheres with surface ligands to deliver drugs or gene-modifying agents directly to the vulnerable plaque itself.

When all is said and done, the thorough understanding of etiology, mechanism, identification, and treatment of atherothrombosis still remains an elusive goal. It is our hope that this issue of Dialogues will provide a concise and exhaustive summary of where we currently stand and what new therapies we can expect to prevent acute myocardial infarction and sudden cardiac death and prevent the “landslide” released by the vulnerable plaque from exacting its deathly toll.
The vulnerable plaque in the natural history of atherosclerosis

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Experimental models of atherogenesis have provided a growing body of information about molecular mechanisms of plaque growth; however, transition from coronary stability to instability is less well understood due to a lack of animal models reflective of human disease. The abrupt clinical presentation of acute coronary syndromes (ACS) gives a strong signal of discontinuity in the natural history of atherothrombosis. The causes of such discontinuity are complex, probably multiple, and still largely unknown. When primary prevention of atherosclerosis fails, the progression of coronary atherosclerosis can remain clinically silent for years, decades, or even for life as indicated by the high prevalence of coronary atherosclerosis in subjects dying of noncardiac causes. In contrast, some patients, at a certain point in their life exhibit ACS, followed by a period of stability, which can be short or last for years or decades. These simple observations suggest that the mechanisms responsible for plaque growth and for plaque instability are different. A better knowledge of the causes of coronary instability might allow new therapeutic targets to be identified that are aimed at the preservation of plaque stability in those subjects in whom primary prevention fails to prevent plaque growth. The goal of this review is to offer a revisitaton of the concept of the "vulnerable plaque" in the light of a pathogenetic classification of ACS that might help in the search of new diagnostic algorithms and therapeutic targets.

Keywords: acute coronary syndromes; atherosclerosis; inflammation; microcirculation; pathogenesis; vasospasm

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SELECTED ABBREVIATIONS AND ACRONYMS

| ACS | acute coronary syndromes |
| AMI | acute myocardial infarction |
| CAD | coronary artery disease |
| CRP | C-reactive protein |
| IL | interleukin |
| IFN | interferon |
| MMPs | metalloproteinases |
| MPO | myeloperoxidase |
| OCT | optical coherence tomography |
| PMN | polymorphonuclear neutrophil |
| TF | tissue factor |
| TGF | transforming growth factor |
| TLR | toll-like receptor |
| TNF | tumor necrosis factor |
lesions. These simple clinical observations suggest that the mechanisms responsible for plaque growth and for plaque instability are different and that the causes and mechanisms of plaque instability are multiple. Accordingly, the paradigm, which implies that a single type of culprit coronary plaque is a cause for instability does not adequately fit the findings of post-mortem studies. Indeed, plaque fissure is frequently asymptomatic and contributes to stepwise, clinically silent, plaque growth rather than precipitating an abrupt coronary occlusion. Also, plaque fissure is not observed in 30% to 50% of patients with ACS. Postmortem studies and recent intravascular imaging have shown that ACS can be associated with plaque rupture, plaque erosion, or a smooth plaque. Furthermore, plaque composition leading to ACS is heterogeneous. The notion that inflammatory cell activation plays a key role in the pathogenesis of ACS was promptly accepted by the scientific community and it is now commonly believed that activation of inflammatory cells in the culprit stenosis is the cause of coronary instability in all patients. Yet, this notion is in sharp contrast with the observation that approximately 40% of patients with ACS have low or very low levels of C-reactive protein (CRP), a very sensitive marker of inflammation. Finally, coronary angiography fails to demonstrate obstructive atherosclerosis in up to one-third of patients with symptoms suggestive of ACS, increased troponin levels, and/or ischemic-like ST segment changes, thus suggesting that functional alterations of epicardial arteries and/or coronary microcirculation play an important pathogenetic role.

THE VULNERABLE PLAQUE IN THE PATHOPHYSIOLOGY OF ACS

In the mid-nineties it became clear that plaque quality rather than plaque quantity was more likely to explain coronary instability. Indeed, milestone postmortem studies demonstrated that the most obvious features distinguishing patients with ACS from those with stable coronary artery disease (CAD) were plaque fissure, presence of a fresh thrombus, and presence of inflammatory infiltrates, typically occurring at the site of mild coronary stenosis. Plaques characterized by positive remodeling, large central lipid core, large number

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**Figure 1.** Coronary lesions in acute coronary syndromes as assessed by multislice computed tomography. CT characteristics of a LAD culprit lesion in a patient presenting with ACS. A, Coronary angiogram. B, Volume rendering. C, Curved MPR. The white arrows in (A) and (B) show the site of luminal obstruction or the culprit lesion. As shown by the solid yellow arrows at 2 sites in the culprit lesion in (C), the lesion is positively remodeled as compared with the normal coronary segment proximal to the lesion (denoted by an interrupted yellow line). A red line denotes a soft plaque (low attenuation), and pink denotes a fibrous plaque.

**Abbreviations:** ACS, acute coronary syndrome; CT, computed tomography; LAD, left anterior descending artery; MPR, multiplanar reformation.

of inflammatory cells, low number of smooth muscle cells, and a thin fibrous cap were then termed “vulnerable” or “high-risk” plaques. Such findings led to the widely accepted unifying paradigm that instability was the consequence of fissuring of a lipid-rich plaque, vulnerable to mechanical stress, as originally thought, and/or to inflammatory weakening of its collagen structure, as is now emphasized. Accordingly, manifold attempts have been issued to develop methods for “vulnerable” plaque detection, a quest predicated on the postulate that local interventions could preclude plaque thrombosis and possibly prevent ACS (Figures 1, 2).

This paradigm, which implies that a single type of culprit coronary plaque is a cause for instability should be modified to fit the findings of postmortem and clinical studies. Plaque rupture associated with thrombosis is the most frequent plaque feature in patients with ACS. This observation has been obtained in the postmortem setting, but also in vivo by using angioscopy, intravascular ultrasound, and, more recently, optical coherence tomography (OCT). Angiographic studies have shown that patients with ACS typically present complex coronary stenoses, suggestive of disrupted plaques complicated by thrombus formation. Yet, complex stenoses can also be observed in a sizeable proportion of patients with stable CAD. Thus, some complex plaques may undergo remodeling and become smooth, while others may remain angiographically complex, but they eventually become functionally stable. Interestingly, the

![Image of plaque rupture](image)

Figure 2. Intravascular optical coherence tomography in the assessment of coronary plaques. OCT provides a high-resolution imaging method for plaque characterization. A. OCT TCFA lesion that shows regions with low backscattering (yellow arrows) and a thin fibrous cap (red arrow). B. Plaque rupture. Yellow arrow delineates a broken fibrous cap. The contents of the ruptured plaque are partially washed away by the flush, leaving behind a cavity (white arrow). C. Plaque erosion. A white thrombus (white arrow) is present on an irregular luminal surface. There is no evidence of rupture. D. Red thrombus. Yellow arrow points to a red thrombus protruding into the lumen with high IVOCT backscattering and attenuation. E. White thrombus. White arrow points to a white thrombus with homogeneous backscattering and low attenuation that is attached to the surface of a coronary artery involving stent struts. There is extensive stent strut malapposition in this image. F. Intimal vascularity. Intimal vessels are well-delineated regions or voids with low IVOCT backscattering (yellow arrows). G. Macrophage accumulations. Macrophage accumulations appear as confluent or punctate highly backscattering focal regions within the artery wall (red arrows), which may be more readily appreciated by visualizing the images using an inverse gray scale look-up table. *Guide-wire artifact.

Abbreviations: IVOCT, intravascular optical coherence tomography; OCT, optical coherence tomography; TCFA, thin-capped fibroatheroma.

**Histological Assessment of Coronary Specimens**

Histological assessment of coronary specimens obtained by atherectomy showed evidence of thrombus in patients with stable CAD, although less frequently than in patients with ACS. Even coronary interrogation with intravascular imaging modalities has failed to identify alterations specific for ACS. Indeed, in a recent study, OCT identified plaque rupture in 16% of patients with stable angina, while it failed to identify plaque rupture in 33% of patients with ACS. In another study carried out on a large population of patients who presented with ACS and underwent percutaneous coronary intervention, about half of the major adverse cardiovascular events occurring during 3 years of follow-up were attributable to originally nonculprit lesions. Gray-scale and radiofrequency intravascular ultrasonography analysis during the first angiography demonstrated that only half of the originally nonculprit lesions responsible for events at follow-up were thin-capped vulnerable plaques. Thus, the current morphologic assessment of coronary plaques fails to reliably predict the occurrence of ACS. It is also worth noting that vulnerable plaque rupture may not necessarily lead to ACS, as asymptomatic plaque rupture is a frequent event leading to plaque progression. The reasons why some plaque ruptures lead to ACS while other do not, are largely unknown. Mechanisms leading to plaque rupture may be classified according to levels of systemic markers of inflammation; indeed some patients exhibit marked elevation of inflammatory biomarkers and widespread coronary microvascular and myocardial inflammation with activation of innate and adaptive immunity while others have immeasurable levels of inflammatory biomarkers suggesting mechanisms other than sustained inflammation.

**PATHOGENETIC CLASSIFICATION OF ACS**

The complexity of postmortem and clinical observations suggests that it is unlikely to identify a common cause for the ACS phenotype. In order to get closer to the multiple causes of coronary instability, we propose a pathogenetic classification of ACS based on culprit plaque morphology and systemic evidence of inflammation (Figure 3). Our ACS classification provides a framework for understanding the basic mechanisms responsible for coronary instability rather than a classification for immediate clinical use such as that provided by the universal definition of myocardial infarction; however, it might help in the search for new diagnostic algorithms and therapeutic targets.

**Plaque Rupture with Systemic Inflammation**

Several studies have shown that systemic inflammation, as assessed by CRP, plays a crucial role in the process of coronary instability. In particular, it has been found that inflammatory mechanisms regulate the fragility of the fibrous cap, as well as the thrombogenic potential of the lipid core. Ruptured plaques and vulnerable plaques compared with intact plaques have increased numbers of inflammatory cells, mostly monocyte-macrophages, but also T lymphocytes, eosinophils, and mast cells. These inflammatory cells, which have shown evidence of activation, are mostly located in the shoulders of the fibrous caps and in the lipid core as well as in the adventitia around areas of neovascularization. The latter might contribute to the recruitment of inflammatory cells in atherosclerotic plaques. The three main features of inflammation associated with ACS are the following: (i) widespread involvement of epicardial arteries, coronary microcirculation, and even the myocardium; (ii) activation of innate immunity; and (iii) activation of adaptive immunity.
**Widespread coronary inflammation**

Inflammatory activation is not confined to the culprit stenosis as is suggested by the observation of widespread neutrophil activation in the coronary circulation of patients with unstable angina and by the presence of activated T cells in remote unaffected myocardial regions in approximately two-thirds of patients with a recent myocardial infarction. Moreover, by interrogating all major coronary artery branches by intravascular ultrasounds, Rioufol et al observed multiple ruptures in patients with a first ACS. Widespread acute coronary inflammation is, therefore, the likely cause of multiple complex stenoses, multiple thrombi, and multiple fissured plaques involving different coronary artery branches as has been observed in clinical studies on ACS that were based on angiography and intravascular imaging. Of note, the number of disrupted coronary plaques correlates with systemic high sensitivity CRP levels.

**Activation of innate immunity**

Among cells of the innate immunity, neutrophils and macrophages play a major role. Interestingly, the activation of neutrophils in the coronary circulation is suggested by telomerase activation in neutrophils from the culprit coronary plaques of patients with ACS, but not in neutrophils from plaques of patients with stable angina. The re-activation of telomerase, demonstrated in the early phases of coronary instability, delays cell apoptosis thus favoring inflammation persistence. Accordingly, we have recently shown a delayed apoptosis of peripheral polymorphonuclear neutrophils (PMN) in ACS patients. Neutrophil activation in ACS appears to be an early and short-lasting event.

The predominant inflammatory cells in atherosclerotic plaques are macrophages recruited as monocytes from circulating blood. There are different subsets of monocytes with different gene expression patterns and, in particular, differential expression patterns of CD14 and CD16. The amount of CD14+CD16+ monocytes in patients with coronary atherosclerosis is higher when compared with healthy subjects, and peak levels of CD14+CD16+ monocytes after an acute MI negatively correlate with the recovery of left ventricular ejection fraction (LVEF) 6 months after MI. Of note, noninvasive imaging technologies have been developed to exploit the functional differences in phagocytic activity between monocyte subsets in order to follow and quantify monocytes subpopulations in patients. Moreover, macrophages are probably involved in the rupture of the fibrous caps as they produce larger amount of matrix degrading metalloproteinases (MMPs), enzymes that degrade all components of the extracellular matrix. The production and activation of MMPs is regulated at the gene transcription level and by the secretion of tissue inhibitors of MMPs (TIMPS). Thus, increased gene transcription of MMPs or reduced activity of TIMPS can enhance matrix proteolysis. It has been shown that toll-like receptors (TLR) may mediate the activation of monocytes and macrophages, indeed monocytes accumulated within thrombi, obtained during primary percutaneous coronary interventions, specifically overexpress TLR4, together with specific patterns of locally expressed chemokines and cytokines compared with circulating monocytes. Notably, Niessner et al has confirmed that TLR4 expression is enhanced in carotid plaques, probably mediated by interferon (IFN)-α released by plasmacytoid dendritic cells (cells that are specialized in sensing danger signals from bacteria and tissue breakdown). Thus, all components leading to the activation of the MMP pathway are present in atherosclerotic plaques. A recent study by Blair et al demonstrated that human platelets also express functional TLRs. In particular, TLR2, in particular, promotes platelet-leukocyte interactions that amplify platelet derived inflammatory signals. In this perspective, platelets act as coprotagonists in plaque activation and as a major player in the thrombotic processes. Moreover, Beaulieu et al has shown that TLR2 is expressed on megakaryocytes and suggested that inflammation, through TLR2 stimulation, can increase megakaryocyte maturation and modulate the megakaryocyte phenotype, potentially influencing platelet function and thrombosis.

**Activation of adaptive immunity**

The higher systemic frequency of activated T cells in patients with ACS compared with stable angina patients, and the higher prevalence of oligoclonal T-cell expansion in unstable coronary plaques compared with stable plaques suggests that the sudden change leading to coronary instability might be related to mechanisms involving adaptive immunity. We have consistently observed that patients with ACS have an increased frequency of auto-aggressive CD4+ T cells that are characterized by a defective cell surface expression of CD28, a major costimulatory molecule critically involved in determining the outcome of antigen recognition by T cells. In ACS, CD4+CD28null T cells are increased in the peripheral blood and infiltrate unstable coronary plaques where they undergo clonal expansion, probably triggered by specific antigens. They release large amounts of pro-inflammatory cytokines, in particular IFN-γ, which activate mono-
Pathogenesis of acute coronary syndromes - Crea and Liuzzo

Both innate immunity (A) and adaptive immunity (B) play a key role in the pathogenesis of coronary plaque instability. A. All types of inflammatory cells are present in atherosclerotic plaques. Macrophages and mast cells infiltrate the lesion and are particularly abundant in the shoulder region where the atheroma grows and where the risk of plaque rupture is higher. B. T-cell infiltrates are always present in atherosclerotic lesions and their activation might play a primary role in the transition from stable to unstable plaques. Such infiltrates are predominantly CD4+ T cells, which recognize protein antigens (such as ox-LDL, human HSP-60, and chlamydial proteins) that have been processed and presented by activated APCs. Recently, the attention has been focused on the possible role of Th17 cells, which are known to play critical roles in the development of autoimmunity and allergic reactions by producing IL-17, and to a lesser extent, TNF-β and IL-6. Another subset of Th1 cells in the plaque have the CD4+CD28null phenotype. These T cells have important plaque-destabilizing properties. Treg cells maintain the homeostasis of cell subsets involved in adaptive immunity. In human atherosclerotic lesions, Treg cells correlated with IL-10 and TGF-β1 expression.

Abbreviations: Ag, antigen; Ang I, angiotensin I; APC, antigen presenting cell; CCR5 and CCR7, chemokine receptors; EC, endothelial cell; EDRF, endothelium derived relaxing factor; ET1, endothelin 1; HOCI, hypochlorous acid; HSP-60, heat shock protein 60; IFN-γ, interferon-γ; IL, interleukin; IL-12R, IL-12 receptor; KIR, killer immunoglobulin like receptor; LTB-4, leukotriene B-4; M-CSF, macrophage colony stimulating factor; MIF, migration inhibitory factor; MMPs, metalloproteinases; MØ, macrophage; MPO, myeloperoxidase; MP-TF+, tissue factor bearing microparticles; ox-LDL, oxidized-low density lipoprotein; PAF-4, platelet activated factor-4; PAI-1, plasminogen activator inhibitor-1; PELT, platelet; PMN, polymorphonuclear neutrophil; ROS, reactive oxygen species; sCD40L, soluble CD40 ligand; SMC, smooth muscle cell; TF, tissue factor; TGF-β, transforming growth factor-β; Th0, Th0 helper cell; Th1, Th1 helper cell; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-α; TRAIL, TNF-related-apoptosis-induced ligand; Treg, regulatory T cells. Modified after reference 30: Crea and Liuzzo. J Am Coll Cardiol. 2013;61:1-11. © 2013, American College of Cardiology Foundation.
cytes and macrophages, have direct cytolytic effects on endothelial cells (amplified by high sensitivity CRP), and vascular smooth muscle cells (VSMCs). VSMC apoptosis has been implicated in the destruction of the plaque surface. By directly stimulating VSMC apoptosis or by coordinating and activating macrophages to kill these cells through the elevated production of IFN-γ, CD4+CD28null T cells could weaken the fibrous cap and destabilize angiogenic vessels, precipitating atherosclerotic plaque rupture. Moreover, CD4+CD28null T cells, either isolated from plaque tissue or from the peripheral blood of patients with ACS, spontaneously express the interleukin (IL)-12 receptor (IL-12R) and respond to IL-12 released by innate immune cells with the upregulation of chemokine receptors, thus, IL-12 can favor their tissue homing even in the absence of antigenic stimulation. Alternative costimulatory molecules regulate CD4+CD28null T cells and their inflammatory and cytotoxic function can be inhibited by blocking these costimulatory receptors. We have recently shown that high frequencies of CD4+CD28null T cells increase the risk of ACS, particularly in diabetic patients.

Two other T cell subsets, type 17 helper T cells (Th17) and CD4+CD25+ regulatory T cells (Treg), have been found to be profoundly perturbed in ACS. Th17 cells expressing retinoic acid-related orphan receptor gamma t play critical roles in the development of autoimmunity and allergic reactions by producing IL-17 and, to a lesser extent, tumor necrosis factor β (TNF-β) and IL-6. Although the precise role of IL-17 in atherosclerosis remains controversial, recent experimental studies in mouse models have provided direct evidence that IL-17 is predominantly proatherogenic. Treg cells expressing the forkhead/winged helix transcription factor (Foxp3) have been found to prevent atherosclerosis in mouse models. The normal function of Treg cells may be essential to maintain the homeostasis of cell subsets involved in adaptive immunity, including antigen presenting cells and effector T cells, by contact-dependent suppression or by releasing anti-inflammatory cytokines (eg, IL-10 and transforming growth factor [TGF]-β1). Consistently, a critical role for the anti-inflammatory cytokine IL-10 has been assumed in Treg cell mediated atheroprotection, both in experimental models and in human atherosclerotic lesions, where the presence of Treg cells correlated with IL-10 expression. The balance between Th17 cells and Treg cells may be important in the development/prevention of inflammatory and autoimmune diseases. Recently published data demonstrated that the Treg compartment is defective in ACS. The number and the suppression efficiency of Treg cells were reduced in patients with ACS as compared with patients with stable angina and healthy controls. A parallel increase in the circulating levels of Th17 cells has also been observed. Taken together, these findings support the notion that, at least in a subset of ACS patients, the failure to mount a counter regulatory response to the activation of aggressive effector T cells might play a key pathogenetic role and represent an attractive therapeutic target.

The presence of activated T cells in ACS implies antigenic stimulation. Indeed, T cells specific for antigens like Chlamydia pneumonia, heath shock proteins, or oxidized low density lipoproteins (ox-LDL) have been isolated from atherosclerotic plaques of ACS patients.

Potential triggers of inflammation associated with ACS

The presence of activated T lymphocytes in ACS strongly suggests antigenic stimulation. Infectious agents like C pneumonia and Helicobacter Pylori have been associated with the risk of CAD in several epidemiological studies, although other studies have reported negative findings (reviewed in refs 79,80). C pneumonia has been detected in advanced coronary atherosclerotic lesions and in vitro investigations have shown that C pneumoniae is capable of infecting vascular endothelial cells and smooth muscle cells and thereby initiates inflammatory activation of these cells via the nuclear factor κB (NF-κB) pathway, resulting in increasing expression of adhesion molecules, inflammatory cytokines, and tissue factor (TF). Moreover, antigen mimicry between the chlamydial and the human heat shock protein (HSP)-60 (85% homology), can activate autoreactive T cells. Interestingly, it has been demonstrated that >50% of the CD4+CD28null T-cell clones derived from patients with ACS recognize human HSP-60. More recently, we have found that one-third of patients with ACS and none of those with stable angina harbor C pneumoniae in plaque tissue obtained during directional coronary atherectomy of the culprit lesion, and this was associated with systemic evidence of active C pneumoniae infection.

The prevalence of infection by more virulent strains of H pylori, those that contain the cytotoxin-associated gene-A (CagA), was found to be higher in patients with ischemic heart disease than in healthy controls. In a meta-analysis, CagA seropositivity was strongly associated with the occurrence of ACS. Notably, it has been demonstrated that anti-CagA antibodies recognize antigens localized inside the atherosclerotic plaque.
Thus, in a subset of patients with ACS, an intense immune response against CagA-positive *H. pylori* strains might precipitate coronary instability mediated by antigen mimicry between the CagA antigen and a protein contained within the plaque.

In addition, pathophysiological observations, epidemiological findings, and clinical findings suggest that acute infections, especially respiratory, might trigger ACS.\(^{84,85}\) Accordingly, randomized trials have shown that vaccination against influenza is associated with a lower rate of acute coronary events.\(^{84,86}\) Acute infections, in addition to eliciting systemic inflammatory responses, can also have direct inflammatory effects on atherosclerotic plaques.\(^{80}\)

Several autoantigens expressed in the atherosclerotic plaque are able to elicit an immune response, including ox-LDL. Yet, in a recent study, we observed that the percent of the plaque area occupied by ox-LDL was similar in patients with either chronic stable angina or ACS.\(^{87}\)

### Plaque rupture without systemic inflammation

When plaque rupture occurs in the absence of systemic inflammatory activation other mechanisms are likely to play a key pathogenetic role, and include extreme emotional disturbances, ranging from external events of short duration (e.g., earthquakes and a beloved team’s loss of a football match) to acute manifestations of more long-lasting internal emotional dispositions, intense physical exertion, as well as local mechanical stress at the level of the artery wall (both circumferential stress and shear stress).\(^{30}\) In addition, subclinical inflammation in the culprit stenosis microenvironment may play a role in the chain of events leading to coronary instability, although the triggers and mechanisms of inflammation are probably different from those operating in patients with systemic evidence of inflammation. It is worth noting that while in the latter group of patients, a large number of studies have clarified the molecular mechanisms leading to coronary instability, however, patients without systemic evidence of inflammation have been investigated less extensively and, consequently, the precise causes of instability are still poorly known, thus providing a strong stimulus for further research in this fascinating setting.

The ability of systemic stress to induce plaque rupture is related to sympathetic nervous system activation and catecholamine release associated with increases in heart rate, blood pressure, and coronary vasoconstriction favoring the rupture of vulnerable plaques\(^{88}\) and is related to platelet activation, hypercoagulability, and intense coronary microvascular constriction.\(^{89}\) Obviously, physical or emotional stress per se is insufficient to cause coronary instability, but it might trigger instability in the presence of thin-capped plaques, causing a severe stenosis.\(^{28}\) Indeed, under these circumstances, stress can cause mechanical rupture of the thin cap while thrombus formation is favored by the prothrombotic effects of systemic stress as well as by the increased shear stress.\(^{90,91}\) Accordingly, Burke et al found a higher prevalence of plaque fissures among patients dying of sudden coronary death during intense stress compared with patients in whom death occurred at rest. Stress-related plaque fissures exhibited a thinner cap compared with plaque fissure occurring at rest, suggesting greater susceptibility to biomechanical forces.\(^{92}\)

Changes in plaque composition have been hypothesized by Abela et al\(^{93}\) as a possible cause of plaque rupture (Figure 5). Indeed local changes in pH, temperature, cholesterol saturation, and hydration promote cholesterol crystallization in the lipid core, which is associated with a quick volume expansion potentially causing plaque fissure and thrombosis. These mechanisms may be amplified by crystallization of free cholesterol from erythrocyte membranes when intraplaque hemorrhage occurs.\(^{94,96}\) The development of micro-OCT with a 2 μm resolution will probably shed new light on this potential mechanism of instability by allowing an in vivo assessment of its occurrence.\(^{97}\) Interestingly, it has recently been shown that human monocytes and macrophages avidly phagocytose cholesterol crystals resulting in a dose-dependent secretion of mature IL-1β, a potent proinflammatory cytokine, through an inflammasome-mediated pathway.\(^{98,99}\) This mechanism may represent an important link between cholesterol metabolism and local inflammation in the microenvironment of atherosclerotic lesions. In this setting, however, the causes of inflammation are likely to be different from those operating in patients with systemic evidence of inflammation.

### Plaque erosion

Plaque erosion is reported in at least one-third of patients dying of acute myocardial infarction (AMI) in postmortem histopathological studies.\(^{2}\) If there is no continuity between the thrombus and the necrotic core and if the thrombus is in direct contact with the fibrointimal plaque, then coronary instability is assumed to...
be the result of plaque erosion. Upon OCT analysis, plaque erosion shows evidence of thrombi, an irregular luminal surface, and no evidence of cap rupture evaluated in multiple adjacent frames (Figure 2C).

Neutrophil activation seems to play a pivotal role in plaque erosion. We have recently shown that patients presenting with ACS associated with plaque erosion had higher systemic myeloperoxidase (MPO) levels as compared with levels in patients exhibiting plaque rupture. Moreover, in postmortem coronary specimens, luminal thrombi superimposed on eroded plaques contained a much higher density of MPO-positive cells than thrombi superimposed on ruptured plaques (Figure 6, page 14). One study reported an intense immunostaining pattern for hyaluronan and its receptor, CD44, along the plaque/thrombus interface in eroded plaques, but not in fissured or stable plaques.

Accumulation of hyaluronan and expression of CD44, along the plaque/thrombus interface in eroded plaques, may promote de-endothelialization, resulting in CD44-dependent platelet adhesion and subsequent thrombus formation, mediated in part by a direct action of hyaluronan on fibrin polymerization. Furthermore, accumulation of hyaluronan in eroded plaques may promote CD44-dependent adhesion and accumulation of circulating neutrophils and MPO-expressing monocytes, which in turn, may enhance endothelial cell death and promote thrombus formation. MPO, released by neutrophils, catalyzes the formation of MPO-derived reactive species (MDRS) such as hypochlorous acid (HOCl), using chloride, thiocyanate, or nitric oxide (NO) as a substrate and hydrogen peroxide as a co-substrate. MDRS are responsible for consuming NO, which may result in impaired vasodilation, oxidation of LDL, high-density lipoprotein (HDL), proteoglycans, and glycosaminoglycans, activation of MMPs, and apoptosis of endothelial cells by activation of specific pathways. Moreover, activated neutrophils shed microparticles, which may transfer TF into platelets thus contributing to thrombosis. TF expression and activation is also induced by MDRS and ox-LDL. MPO may also have a role in thrombus growth.

Notably, the traditional belief that coagulation activation may be initiated and propagated only by contact of circulating blood with the extravascular space when the vessel is damaged, has recently been challenged by detection of TF, the key initiator of the coagulation cascade, in the bloodstream. Indeed, TF is expressed on the surface of circulating cells, such as leukocytes and platelets, as a functionally inactive form (a phenomenon defined as “encryption”) whose activity can rapidly increase in response to specific stimuli without the need for de novo protein synthesis (“decryption”). Furthermore, TF circulates as a component of cell-derived microparticles originating from endothelial cells, vascular smooth muscle cells, leukocytes, or platelets, both when the cells are activated or undergo apoptosis. TF containing microparticles are also released from atherosclerotic plaques. Finally, TF circulates in the
bloodstream as a soluble protein lacking the transmembrane domain generated by an alternatively spliced mRNA (“alternatively spliced TF”). The importance of circulating vs vessel wall-associated TF in thrombus formation and growth is still a subject of debate. Finally, calcified nodules, found to be more frequent in patients with diabetes,\textsuperscript{107} are a less common cause of coronary instability. They are lesions with the highest concentration of calcification relative to plaque area and can be a rare trigger for thrombosis.\textsuperscript{2} 

**Functional alterations of epicardial coronary arteries**

Plaque fissure has recently been observed in women with ACS and without obstructive atherosclerosis, which potentially causes transient thrombus formation.\textsuperscript{108} Yet, its pathogenic role is difficult to establish because plaque fissure is frequently asymptomatic and is observed in a sizeable proportion of patients with stable ischemic heart disease.\textsuperscript{25} Epicardial coronary

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**Figure 6. Different culprit plaque morphologies may subtend different pathogenetic mechanisms.**

Histological analysis of culprit plaques from sudden coronary deaths showed a higher density of MPO-positive cells in luminal thrombi superimposed on plaque erosion than in thrombi superimposed on plaque fissure. 1A and 1B. are images of plaque erosion and plaque fissure with occlusive thrombus, respectively (modifiedMovat pentachrome). 1A. Plaque erosion over a lesion exhibiting a deep lipid pool. 1B. The yellow arrow shows the rupture site with a luminal thrombus in communication with a large necrotic core. 2A-4B. Represent serial images within the area of the thrombus in 1A and 1B, respectively, 2A and 2B. Immunostaining for MPO (brown reaction pigment); the density of myeloperoxidase-positive cells in the luminal thrombus in erosions is notably higher than in the fissure. 3A and 3B. Recognition of neutrophils by cathepsin G immunostaining, which was greater in erosions than in the fissure. 4A and 4B. Higher magnification images dual-stained for MPO (red) and the macrophage marker CD68 (blue). Although not exclusive, MPO expression was also present in macrophages. C. Density of MPO-positive cells within the thrombus overlying eroded or fissured plaques. D. Density of MPO-positive cells in the area of the fibrous cap in eroded or fissured plaques. Box plot represents median, interquartile range, minimum, and maximum values.

**Abbreviations:** Lp, lipid pool; MPO, myeloperoxidase; NC, necrotic core; Th, thrombus.

vasospasm is likely to play a key role in ACS, particularly in patients in whom coronary angiography fails to demonstrate the presence of an obstructive atherosclerotic plaque. In the CASPAN study (Coronary Artery Spasm in Patients with Acute Coronary Syndrome), coronary angiography failed to show obstructive atherosclerosis in about 30% of patients with suspected ACS. More importantly, intracoronary acetylcholine administration elicited coronary spasms in nearly 50% of these patients.11

Coronary spasms are caused by vasoconstrictor stimuli acting on hyperreactive smooth muscle cells.109 It can occur at the site of an angiographically normal coronary segment or in the presence of a nonobstructive atherosclerotic plaque. The molecular causes of smooth muscle cell hyperreactivity are still incompletely understood. As several vasoconstrictor stimuli acting on different unrelated receptors are able to precipitate coronary spasm in the same patient, smooth muscle cell hyperreactivity, rather than by an abnormality of a single receptor, might be caused by ionic pump alterations, such as an enhanced activity of the sodium ion/hydrogen ion exchanger, eventually leading to intracellular calcium overload. More recently, several experimental and clinical studies have shown that enhanced light myosin chain phosphorylation by rho kinase, which makes myosin more sensitive to intracellular calcium, might play a key role in the pathogenesis of coronary spasms.110 Unpublished data by our research group show that ACS patients with smooth plaques without thrombus at OCT interrogation have increased cystatin C levels in agreement with data by Funayama et al showing that cystatin C levels are elevated in patients with vasospastic angina.111

**Functional alterations of coronary microcirculation**

Intense constriction of coronary microcirculation is a second mechanism that may cause ACS in patients who exhibit nonobstructive coronary atherosclerosis.112,113 This is the likely mechanism of instability in patients with Takotsubo syndrome, which is characterized by ischemic pain at rest, ST segment elevation, cardiac enzyme release, and a characteristic regional akinesia more frequently affecting distal myocardial regions associated with hypercontractility of the remaining regions. Indeed, in these patients, using echocontrastography, we have recently found regional apical hypoperfusion, which transiently improves during administration of adenosine, a potent arteriolar vasodilator. This transient improvement of perfusion during adenosine administration is associated with a transient improvement of regional wall motion abnormalities and ejection fraction (Figure 7, page 16).114

Thus, reversible coronary microvascular dysfunction seems to be the common pathophysiological determinant of this syndrome. The causes of this intense microvascular constriction and the reasons for its peculiar locations are still largely unknown. Sympathetic hyperreactivity and myocarditis are potential, yet unproved, causes. The Parvovirus B19 genome has been identified in myocardial biopsies of patients with angiographically normal coronary arteries, presenting with typical ischemic pain, ischemic ST segment changes, cardiac necrosis enzyme increases, and various degrees of regional wall motion abnormalities.115 Its presence in the myocardium was associated with coronary vasoconstriction in response to acetylcholine, thus suggesting severe endothelial dysfunction.116

Finally, lower intensity microvascular constriction that occurs in the absence of regional wall motion abnormalities and is associated with an unstable pattern of angina, ischemic ST segment changes, and/or cardiac necrosis enzyme increase has recently been proposed as the unstable counterpart of stable microvascular angina, which is more prevalent in women than in men.117 According, the prevalence of women who present ACS in the absence of obstructive atherosclerosis is 2-fold higher than that of men.117 The mechanisms of this poorly defined presentation of coronary instability are unknown. Functional alterations of coronary microcirculation, similar to mechanisms described in women with stable microvascular angina, might be involved.118 There are a number of likely causes for coronary flow reserve impairment in patients with nonobstructive atherosclerosis. Coronary flow is regulated by several endothelium-dependent and independent factors influencing microvascular tone. Endothelium-independent factors include aortic pressure, myocardial compressive forces, neurohumoral substances, and myocardial metabolism. The endothelium regulates vasomotor tone by stimulating release of vasoactive factors. A major vasodilator substance is nitric oxide, originally identified as an endothelium-derived relaxing factor. In the WISE study (Women’s Ischemia Syndrome Evaluation), coronary microvascular reactivity to adenosine predicted an adverse outcome in women evaluated for suspected ischemia.119,120

Finally, it has recently been proposed that epicardial or microvascular spasms might promote coronary thrombosis at the site of a susceptible plaque, thus po-
tentially representing the primary cause of instability, also in some of the patients exhibiting obstructive atherosclerosis (Figure 3).\textsuperscript{121}

**CLINICAL PERSPECTIVES**

The large body of knowledge we have gained in the past 50 years in the field of cardiovascular diseases clearly indicates that our main goal is primary prevention. Indeed, more than 90% of acute vascular events are explained by environmental causes that can theoretically be eradicated to prevent plaque growth.\textsuperscript{122} Thus, it is extremely important to reduce the burden of cardiovascular risk factors. It is equally important, however, to improve our knowledge of the complex mechanisms responsible for the sudden transition from coronary stability to instability. Indeed, when we fail with plaque growth prevention, a subordinate goal is plaque stability preservation.

Coronary thrombosis is the final common pathway leading to coronary instability and it is our current main therapeutic target.\textsuperscript{123} This has allowed us to considerably improve the outcome of ACS, however, more potent antithrombotic regimens have recently been found to increase the risk of major bleedings, which are associated, in turn, with a higher risk of mortality.\textsuperscript{124} In order to identify new therapeutic targets we need to know more about the different causes of coronary thrombosis. Table I and Figure 3 summarize pathogenetic mechanisms of ACS along with diagnostic and therapeutic options tailored to individual mechanisms of coronary instability.

### Plaque rupture with systemic inflammation

Several studies have shown that patients with ACS in whom obstructive atherosclerosis is associated with increased CRP levels or of other inflammation markers have a worse outcome than patients with a similar severity of coronary atherosclerosis, but normal levels of inflammatory markers.\textsuperscript{9,36,40,41,125,126} Thus, reassessment of the inflammatory status after discharge may help identify patients at higher risk of coronary instability recurrence. A change of focus from soluble inflammation markers to adaptive immune activation markers might prove to be more rewarding, as T cells are the main conductors of immune responses and their assessment might allow the identification of new

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**Figure 7. Functional alterations of coronary microcirculation may cause an acute coronary syndrome in patients without obstructive coronary atherosclerosis.**

Reversible intense coronary microvascular dysfunction has been demonstrated in Takotsubo or apical ballooning syndrome. A series of patients with apical ballooning syndrome underwent myocardial contrast ECO at baseline, during adenosine infusion (140 μg/kg/min), a potent coronary microvascular vasodilator, and at a 1-month follow-up. During adenosine challenge, all patients exhibited a significant improvement in myocardial perfusion and LV function, which further improved at the 1-month follow-up. \textbf{A.} A clear perfusion defect is present at baseline within the LV apical myocardium (arrows). \textbf{B.} A striking decrease in the extent of the perfusion defect is evident during adenosine infusion (arrows). \textbf{C and D.} Values of contrast score index and LVEF in individual patients are shown at baseline, at the peak of adenosine infusion, and at a 1-month follow-up, respectively. *P<0.001 vs baseline. Abbreviations: ABS, apical ballooning syndrome; ECO, echocardiography; EF, ejection fraction; IX, left ventricular.

therapeutic targets in the subset of patients in whom an inflammatory outburst is the likely cause of coronary instability. Although the assessment of the inflammatory status is currently based on biomarkers only, recently developed imaging techniques that are able to monitor inflammatory cell activity in atherosclerotic plaques might prove to be more predictive than biomarkers (Figure 8, page 18).

An unmet need in this patient subset is a specific anti-inflammatory treatment, based on the modulation of both innate and adaptive immunity. While non-steroidal anti-inflammatory drugs have been associated with a higher risk of cardiovascular events, probably related to the inhibition of prostacyclin synthesis in endothelial cells, disease modifying antirheumatic drugs have been found, in observational studies, to be associated with lower cardiovascular risk. Accordingly, in the CIRT trial (Cardiovascular Inflammation Reduction Trial), Ridker et al will test the potential beneficial effects of low dose azathioprine in patients with a recent myocardial infarction and high sensitivity CRP levels >2 mg/L. Steroids have successfully been tested in the prevention of clinical restenosis following bare metal stent implantation, but their utilization is hampered by the well-known side effects, in particular, in diabetic and hypertensive patients. Antagonism of key cytokines like TNF-α is another potential approach, particularly in high-risk patients with high levels of CD4+CD28null T cells, although the high cost and the potential side effects make this approach difficult to pursue. Interestingly, statins have been found to modulate this aggressive subset of T cells, which might help explain the early beneficial effect of intensive statin treatment in patients with a recent ACS. Antagonists of IL-1β and IL-1R or inhibitors of inflammasome activation are close to clinical testing. A recent pilot study in patients with AMI has provided encouraging results, showing that IL-1 blockade with anakinra, a recombinant human IL-1R antagonist, is safe and favorably affects cardiac remodeling after AMI, without modifying either the final infarct size or infarct healing. Recently, at least two additional IL-1-targeted drugs have been developed, including a recombinant protein with high affinity for IL-1β, known as IL-1 TRAP, or rilonacept, and a fully humanized anti-IL-1β monoclonal antibody, canakinumab. These drugs have proven effective in several chronic inflammatory diseases, including rheumatoid arthritis and diabetes, and might be of potential interest in ACS. Other ways to limit IL-1β activation are to inhibit either caspase-1 or the inflammasome. Among caspase-1 inhibitors, pralnacasan (VX-740) and VX-765 have been tested in clinical trials. Another potential therapeutic target is Treg cells in patients with a decreased number or a depressed function of their Treg cells. Putnam et al have recently shown that these cells can be isolated and expanded ex vivo for the treatment of auto-

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Diagnosis</th>
<th>Potential tailored therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque rupture with systemic inflammation</td>
<td>CRP levels</td>
<td>High dose statins</td>
</tr>
<tr>
<td>1. Innate immunity</td>
<td>T cell subsets</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>• Early neutrophil activation</td>
<td>Functional imaging</td>
<td>IL-1β antagonists</td>
</tr>
<tr>
<td>• Macrophages producing MMPs</td>
<td></td>
<td>Treg expansion</td>
</tr>
<tr>
<td>2. Adaptive immunity</td>
<td></td>
<td>Vaccines</td>
</tr>
<tr>
<td>• CD4+CD28null T-cells expansion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Th17/Treg imbalance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque rupture without systemic inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sympathetic nervous system activation</td>
<td>Anatomical imaging</td>
<td>Statins; PLA₂ inhibitors</td>
</tr>
<tr>
<td>2. Changes in plaque composition</td>
<td></td>
<td>Enhancement of cholesterol efflux</td>
</tr>
<tr>
<td>Plaque erosion</td>
<td>MPO levels</td>
<td>Antithrombotic drugs</td>
</tr>
<tr>
<td>1. Neutrophil/monocyte expressing MPO</td>
<td>Functional imaging</td>
<td></td>
</tr>
<tr>
<td>2. Hyaluronan expressing CD44 with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Direct action on fibrin polymerization</td>
<td></td>
<td></td>
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<td>and platelets adhesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Recruitment of neutrophils and monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional alterations</td>
<td>Functional tests of vasomotility</td>
<td>Nitrates</td>
</tr>
<tr>
<td>1. Epicardial coronary spasm</td>
<td>Rho kinase activity</td>
<td>Calcium antagonists</td>
</tr>
<tr>
<td>• Enhanced Rho kinase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Microvascular spasm</td>
<td>Rho Kinase inhibitors</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Pathogenetic mechanisms, diagnosis, and potential tailored therapy for acute coronary syndromes.

Abbreviations: CRP, C-reactive protein; IL-1β, interleukin 1β; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; PLA₂, phospholipase A₂; Treg, regulatory T cells.
To biomechanical forces. In addition, the development of micro-OCT, which makes it possible to image coronary artery microstructure at a scale that is comparable with histopathology, might shed some new light on the role played by cholesterol crystallization.97

As it is difficult to limit environmental, physical, or emotional triggers, an obvious target in this patient subset is plaque stabilization as achieved by intensive statin treatment 133 Inhibitors of phospholipase A2 represent another class of drugs that might help in plaque stabilization. Indeed, darapladib, an antagonist of lipoprotein-bound phospholipase A2 (PLA2), was found to reduce the size of the necrotic core, as assessed by virtual histology, compared with placebo.136 Yet, a larger trial with clinical end points of varespladib, an antagonist of secretory PLA2, vs placebo has been halted prematurely because of lack of efficacy.137 Accordingly, a Mendelian randomization meta-analysis of 19 general population studies and 10 ACS cohorts concluded that reducing secretory PLA2 is unlikely to be a useful therapeutic goal for preventing cardiovascular events.138 Another important, but still elusive, target in order to promote plaque stabilization is the enhancement of cholesterol efflux.139 Among patients in whom plaque fissure is not associated with systemic inflammation and ACS occurs in the absence of environmental, physical, or emotional triggers, more information must be discovered regarding the mechanisms that modulate cholesterol crystallization, including the inflammasome pathway, which is activated by cholesterol crystals, in order to identify new therapeutic targets.

Plaque erosion

In plaque erosion, MPO is an important bystander and may even play a pathogenetic role. Molecular imaging techniques can visualize MPO in atherosclerotic plaques in carotid arteries, although their utilization is more difficult in the coronary circulation.127,140 Elevated plas-

### Abbreviations

- MMPs, matrix metalloproteinases
- VCAM-1, vascular cell adhesion molecule-1


**Figure 8. Noninvasive anatomic and functional imaging of vascular inflammation and unstable plaques.**

By harnessing the advances in cardiovascular biology, imaging has advanced beyond its traditional anatomical domains to generate a tool that permits probing of particular molecular structures to image cellular behavior and metabolic pathways involved in atherosclerosis. Several potential molecular and micro-anatomical targets for imaging with selective imaging probes and a variety of imaging modalities have emerged from preclinical and animal investigations. The diagram on the left depicts a plaque in the carotid bifurcation. The expanded view in the middle, illustrates the composition of the plaque (lumen to the right), showing a calcified region in blue at the base of the plaque on the left, macrophages in the lipid rich necrotic core (yellow), triple helical collagen fibrils, and smooth muscle cells in the plaque’s fibrous cap (brown) underlying the endothelial monolayer. The blue dots represent spotty calcification. The microvessels also penetrate the base of the plaque from the adventitia. Various imaging targets on the major cell types shown are listed on the right.

**Abbreviations:** MMPs, matrix metalloproteinases; VCAM-1, vascular cell adhesion molecule-1.

### Plaque rupture without systemic inflammation

Clinical history of extreme emotional disturbance or intense physical exertion might help to identify the subset of patients presenting plaque rupture without systemic inflammation. Furthermore, anatomical (more than functional) features of the atherosclerotic plaque are important for determining coronary instability. Thus, noninvasive imaging of stress-related plaque fissures might reveal a thinner cap compared with plaque fissures occurring at rest, indicating greater susceptibility to biomechanical forces. In addition, the development

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ma concentrations of MPO might represent an important biomarker for the identification of this subset of patients.\textsuperscript{101}

In patients with plaque erosion, the mechanism of inflammation is probably an intense local thrombogenic stimulus. Thus, in this subset of patients a potent antithrombotic treatment, perhaps based on double antiaggregation and an oral anticoagulant, might be the treatment of choice, but this approach needs to be tested in prospective studies.

**Functional alterations of coronary circulation**

Careful assessment of clinical history and 24- to 72-hour ambulatory electrocardiogram monitoring is usually sufficient to achieve the diagnosis of vasospastic angina, whereas the use of provocative tests of coronary artery spasm (e.g., intravenous ergonovine, intracoronary ergonovine, or acetylcholine administration) is required in about 10% of patients.\textsuperscript{109} Rho-kinase activity in circulating neutrophils is increased and might represent a useful biomarker for diagnosis and disease activity assessment in patients with vasospastic disorders.\textsuperscript{141}

Epicardial and microvascular vasoconstriction is the key therapeutic target when ACS is not associated with obstructive atherosclerosis. Recently, data from clinical trials suggest that the outcome in these patients is, on average, better than that of patients with obstructive atherosclerosis; however, about 10% of patients presenting with ACS in the absence of coronary atherosclerosis have a major cardiac event at a 1-year follow-up.\textsuperscript{142} Although nitrates and calcium antagonists are helpful in patients with vasospastic angina, further efforts are needed to identify the molecular alterations responsible for smooth muscle cell hyperreactivity because a sizeable proportion of patients with vasospastic angina are refractory to standard doses of vasodilators.\textsuperscript{109} Recently, it has been observed that fasudil, a specific rho kinase inhibitor, reduces the rate of coronary spasm episodes in patients with vasospastic angina.\textsuperscript{110} Similarly, further efforts are warranted to unravel the molecular mechanisms responsible for coronary microvascular dysfunction in the Takotsubo syndrome, in myocarditis, and in unstable microvascular angina.

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The Vulnerable Plaque

Expert Answers to Three Key Questions

1. How can the vulnerable plaque be identified?
   

2. What are the invasive approaches to the vulnerable plaque?
   
   B. Meier

3. What are the pharmacological approaches to treat the vulnerable plaque?
   
   K. Fox
How can the vulnerable plaque be identified?

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The precise identification of a vulnerable plaque may have substantial clinical impact since the absolute majority of sudden death cases are related to acute coronary syndromes. Advances in imaging techniques have allowed for the development of numerous invasive and noninvasive tools to investigate coronary atherosclerosis. Contemporary natural history studies of atherosclerosis added information on changes in the morphological and compositional plaque characteristics. The objective of understanding these temporal changes is to predict plaques that are prone to have future events, which will change the way we approach vulnerable plaque treatment and strengthen the concept of plaque passivation. This review article summarizes the current definitions on vulnerable plaque, the recent advances in the study of atherosclerosis, current evidence, and highlights our limitations in understanding plaque evolution and predicting plaque destabilization.

DEFINITIONS

Cardiologists describe the plaque responsible for coronary occlusion and death as a culprit plaque. However, clinicians need a similar term for prospective evaluation, to describe such plaques before an event occurs, so they are called vulnerable plaques.

There are two major types of vulnerable plaques, rupture-prone and erosion-prone.3-5 The prototype of a rupture-prone plaque contains a large and soft lipid-rich necrotic core (>30% of plaque) covered by a thin (thickness usually <65 mm) and inflamed fibrous cap. Associated features include large plaque size, expansive remodeling mitigating luminal obstruction (mild stenosis...
**How can the vulnerable plaque be identified?** - Serruys and others

**Figure 1. Imaging applications for plaque assessment.**

A. IVUS gray scale plaque rupture is represented by a large empty cavity from 11- to 2-o’clock. B. RF-IVUS was used to analyze plaque within the lumen and shows a high necrotic core content (red) adjacent to the lumen. C, D. OCT images showing a thin-capped fibroatheroma (C) and a ruptured plaque with a thrombus (D, white arrow). E, F. The output of the NIRS catheter is illustrated (E, chemogram; F, block chemogram). The yellow-red color-coded map illustrates the probability of the presence of a lipid core (yellow corresponds to high probability and red to low probability). G. Output of a recently developed intravascular magnetic resonance probe (IV-MAR); the images were obtained in vitro from an atherosclerotic iliac artery. The dark areas at 9-o’clock (II, III) and 12-o’clock (IV) indicate the presence of calcific tissue. H, I. Data acquired by a combined IVUS and NIRS catheter showing IVUS cross-sections with the corresponding chemogram obtained from a stented (II) and a nonstented segment (I). The probability of the presence of a lipid-rich plaque is low in the stent segment, but is high in the nonstented segment between 1- and 7-o’clock.

**Table 1. Ability of the available imaging modalities to detect plaque features associated with increased vulnerability.**

The ability of the presented modalities to detect plaque characteristics associated with increased instability is graded as: unable (−), low (+), moderate (++), and high (+++).

<table>
<thead>
<tr>
<th>Plaque characteristics</th>
<th>Thin fibrous cap</th>
<th>Necrotic core</th>
<th>Plaque burden assessment</th>
<th>Positive remodeling</th>
<th>Neo-angiogenesis</th>
<th>Active inflammation</th>
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<tr>
<td>IVUS/RF-IVUS</td>
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<td>++</td>
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**Abbreviations:** CTCA, computed tomography coronary angiography; IV-MRI, intravascular MRI; IVUS, intravascular ultrasound; NIRS, near-infrared spectroscopy; OCT, optical coherence tomography; PET, positron emission tomography; RF, radiofrequency; TRFS, time-resolved fluorescence imaging.

*Modalities are in their initial development, and therefore, the data provided for these techniques are derived from small scale in vivo or histology-based in vitro studies.
by angiography), neovascularization, plaque hemorrhage, adventitial inflammation, and a “spotty” calcification pattern. \(^4\)\(^6\)

The erosion-prone vulnerable plaques are heterogeneous and defined only by their fate (thrombosis; mostly mural) \(^7\). The surface endothelium is missing, but whether it vanished before or after thrombosis remains unknown. No single morphological features have been identified, but in general, eroded plaques with thrombosis are scarcely calcified, rarely associated with expansive remodeling, and only sparsely inflamed. \(^7\) Thus, it remains a challenge to distinguish erosion-prone plaques from stable plaques by imaging. \(^8\)

**INVASIVE IMAGING OF VULNERABLE PLAQUES**

**Intravascular ultrasound (IVUS)**

IVUS was the first invasive modality that allowed imaging of the lumen and vessel wall, quantification of plaque burden, and characterization of its composition. Positive vessel remodeling can readily be evaluated with IVUS. \(^8\)\(^9\)\(^10\) Visual assessment of plaque echogenicity provides semi-quantitative tissue characterization. \(^12\) Calcification can be identified with a sensitivity and specificity of approximately 90%. \(^13\) Large eccentric plaques containing an echolucent center by IVUS were associated with the development of acute coronary syndrome (ACS) in a prospective study. \(^14\) Another feature that can be obtained by IVUS is attenuated plaques, defined as plaques with >30 ultrasonic attenuation of deeper arterial structures despite the absence of bright calcium. In an assessment of 131 native lesions, attenuated plaques have a confluent necrotic core (93.5%) when matched by virtual histology (VH)-IVUS and have a lipid core (90.3%) on the block chemogram. \(^15\) Microbubble contrast-enhanced IVUS can measure activity and inflammation within atherosclerotic plaques by imaging vasa vasorum density, which is considered as a marker for plaque vulnerability. \(^16\) The main limitation of IVUS is its 100 to 150 \(\mu\)m axial resolution. The fibrous cap of a thin-cap fibroatheroma (TCFA) is less than 65 \(\mu\)m in thickness, and therefore, cannot be visualized by IVUS (Figure 1).

**Intravascular ultrasound radiofrequency analysis (RF-IVUS)**

RF-IVUS involves spectral analysis of the IVUS gray-scale data and evaluates different spectral parameters. Different plaque components are assigned different color codes: calcified (white), fibrous (green), fibrolipidic (greenish-yellow), and necrotic core (red) (Figure 1B). \(^17\)

The PROSPECT trial (Predictors of Response to Cardiac Resynchronization Therapy) has been the largest study of the natural history of atherosclerosis and used RF-IVUS to detect anatomical and compositional features associated with an increased risk for a plaque to evolve into a culprit lesion. \(^18\) 697 patients treated for an ACS underwent RF-IVUS postintervention at the 3-year follow-up. \(^19\) 104 new symptomatic lesions became manifest in nontreated segments. Multivariable analysis demonstrated that the presence of TCFA, a minimum lumen area ≤4 mm\(^2\), and a plaque burden ≥70% were associated with future events.

Similar results were reported by the VH-IVUS in the Vulnerable Atherosclerosis Study that had a similar design. \(^19\) The PROSPECT trial not only showed the potential predictive value of intravascular imaging, but also highlighted its limited prognostic accuracy as only 4% of the detected TCFA evolved into culprit lesions. This should be attributed to both the fact that the included patients were on optimal treatment and the inherent limitations of IVUS imaging. \(^20\)

**Optical coherence tomography (OCT)**

OCT is an optical ultrasound analogue, however, it uses light instead of sound to create an image. It can provide a resolution of 10 to 20 \(\mu\)m in vivo. This resolution permits visualization of details that cannot be imaged by other intravascular techniques, such as the evaluation of fibrous cap thickness, detection of macrophages, neovascularization, and identification of plaque erosion (Figure 1C, 1D). \(^21\) A limitation of OCT is its poor penetration, which often does not allow complete visualization of the vessel wall or an assessment of vessel remodeling. In addition, the OCT signal cannot penetrate into lipid tissue, and thus, it is unable to quantify the lipid component.

**INVASIVE TECHNIQUES FOR THE DETECTION OF INFLAMMATION**

**Thermography**

Thermography was the first invasive imaging technique developed to identify vessel wall inflammation based on the measurement of plaque heat. High temperatures indicate increased inflammatory activity and sometimes plaque vulnerability. Initial reports demonstrated the efficacy of thermography in detecting high-risk plaques, but recent studies have raised concerns...
How can the vulnerable plaque be identified? - Serruys and others

about its effectiveness in patent coronaries suggesting that blood flow obstruction is necessary to obtain accurate estimations, a fact that has limited its current applications.22-24

Near-infrared fluorescence (NIRF)

NIRF imaging is a novel technique introduced to detect vascular activity. It involves injecting agents that bind molecules related to the plaque's inflammation and have the ability to fluoresce after being irradiated with near-infrared light emitted by a specially designed catheter. Experimental studies demonstrated the feasibility and the potential of this technology.25 Recently, a hybrid NIRF-OCT catheter (diameter 0.8 mm) has been designed that provides simultaneous functional molecular imaging (provided by NIRF) and vessel pathology visualization (given by OCT).26 The feasibility of this approach has been tested both ex vivo and in vivo in animal models and the first results appear promising. However, the safety of this technique has to be proven before being implemented in humans.

Near-infrared spectroscopy (NIRS)

NIRS is based on the principle that different organic molecules absorb and scatter NIRS light to different degrees and wavelengths. Spectral analysis of the obtained signal provides a color-coded display, called a chemogram, which provides the probability that a lipid core is present in the superficial plaque (studied depth =1 mm) (Figure 1E, 1F).

Several studies have examined the reliability of this technique using histology as the gold standard and demonstrated a high overall accuracy in detecting lipid-rich plaques while others demonstrated its feasibility in the clinical setting.27,28 Lipid plaque burden is being used as a predictor of cardiac events in a NIRS sub-study of the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis (NCT01789411).

Further improvements in external coils as well as the development of contrast agents that will allow more accurate plaque characterization are required so that this modality may be useful in this setting.

Noninvasive imaging of vessel wall inflammation

Nuclear imaging constitutes the leading noninvasive modality for the evaluation of vascular activity. Recent reports demonstrated the feasibility of combining CTCA with 18F-FDG imaging for the identification of inflamed plaques on the coronary tree (Figure 2).35,36 The concept of fusing two noninvasive modalities that provide anatomical (derived from CTCA) and biological (given by PET using the 18F-FDG tracer) information constitutes a breakthrough in the study of atherosclerosis because it will allow detailed imaging of plaque pathology in larger populations. This process is expected to provide additional information about the distribution of plaque inflammation and its association with different plaque components.

Apart from 18F-FDG, several other tracers have been developed to assess vascular activity, which have not yet been used in a clinical setting and include: (i) 99mTc-AA5, which binds phosphatidylserine produced by apoptotic cells; (ii) 99mTc matrix metalloproteinase inhibitor that binds active metalloproteinases; and (iii) IK17 tracer, which is labeled with iodine 125 (125I) and detects the presence of oxidized low density lipoprotein.37-39

Future trends and conclusions

Intravascular magnetic imaging (Figure 1G),40 photoacoustic imaging, Raman spectroscopy, and time-
resolved fluorescence spectroscopy are emerging techniques that are still under evaluation and are expected to provide additional information about plaques. In parallel, an effort is being made to overcome the limitations of the prominent intravascular imaging modalities either by developing new methodologies that would allow better processing of the acquired data (eg, focused acoustic computed tomography, micro-OCT, polarized OCT) or by creating hybrid catheters that would permit multimodality intravascular imaging.41,42

A hybrid catheter that combines an IVUS and a NIRs probe (TVC, MC 7 system; InfraRedx, Burlington, MA) is currently available and being used in the research arena.43 Catheters that permit fusion of IVUS with OCT, photoacoustic imaging, or time resolved fluorescence spectroscopy are also under evaluation.44 Initial experimental studies have shown promising results;45-47 however, large dimensions of the available catheters, safety concerns regarding the new techniques, low image acquisition rate, and moderate image quality that they provide have not allow their implementation in humans yet.

These advances are expected to result in a better understanding of the composition and evolution of the atherosclerotic plaque in an attempt to anticipate and prevent acute coronary events.

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How can the vulnerable plaque be identified? - Serruys and others


What are the invasive approaches to the vulnerable plaque?

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In 1977, Gruentzig clinically introduced balloon angioplasty of the coronary arteries, referred to later, in the stent era, as ‘plain old balloon angioplasty’ (POBA). After several thousand POBA procedures, it became apparent that plaques subjected to balloon impact were highly unstable (vulnerable) for a few hours. About 7% of treated arteries occluded acutely in the first 24 hours. It also became apparent that about 30% of the sites that remained patent underwent significant restenosis from tissue proliferation.

Only gradually did it emerge that restenosis was not an indication for emergency reintervention since occlusion at these sites was practically never complete. The explanation is histological. Restenosis usually consists of a thick layer of neoendothelium covering the plaque that has been cracked, dissected, and destabilized by the balloon impact.

The result is impaired flow rather than fissure, erosion, or spontaneous rupture leading to thrombosis and myocardial infarction. De novo stenosis, on the other hand, harbors the risk of rupture of the diseased endothelium, particularly in the presence of a thin-cap fibroatheroma (TCFA), either irrespective of or, at most, barely proportional to, the resulting degree of stenosis. Restenosis, in contrast, can be subtotal yet still practically devoid of the risk of abrupt occlusion and infarction.

These insights prompted the idea of cracking plaques not serious enough to warrant POBA on the grounds of impaired flow, simply to exploit their near-immunity from spontaneous rupture afforded by neoendothelialization. The approach was termed coronary plaque sealing or mechanical plaque sealing. Supporting evidence followed, both clinical and histological, yet ac-
Acceptance was poor. It was surmised that restenosis after mechanical plaque sealing would transform 30% of previously nonsignificant lesions into significant lesions. This did not account for the fact that the restenosis rate had always been lower in mild lesions than in more severe lesions.

A review on the outcome of mild lesions subjected to POBA found that 20% had overall problems during follow-up.\(^7\) This appeared too high a price to pay for trying to prevent an estimated 2% risk of spontaneous plaque rupture per year in interventionally untreated mild lesions. However, investigators should have looked exclusively at the incidence of myocardial infarction or death after POBA of mild lesions, which was 1.6% per year. The remaining problems were coronary interventions that would probably have occurred anyway at some time in patients whose initial treatment was purely medical. Moreover, only lesions with less than 50% diameter stenosis were considered whereas POBA plaque sealing was proposed primarily for lesions with between 50% and 70% diameter stenosis.\(^4\) Most physicians found the increasingly ubiquitous use of statins for the primary and secondary prevention of coronary artery disease as a preferable way to avoid and neutralize vulnerable plaques. This is thanks to their emerging potential not only for modifying lipids, but also for normalizing endothelial function and reducing inflammation and thrombus formation, be it by platelets or fibrinogen.\(^8\)

Figures 1 and 2 show typical cases in which plaque sealing might have been of tremendous clinical and prognostic benefit. In Figure 1 occlusion at the site of interest occurred despite aspirin and statin treatment exactly 1 month after lesion documentation by coronary angiography. The ensuing large anterior myocardial infarction led to heart failure that subsequently required heart transplantation. The message in Figure 2 was more compelling still: outcome was fatal in less than 2 weeks.

### ROLE OF STENTS IN MECHANICAL PLAQUE SEALING

Coronary stents drastically reduced abrupt occlusion after balloon angioplasty, but they introduced acute occlusion in treated sites due to stent thrombosis after hospital discharge. Late occlusion of a dilated site was practically unknown in the POBA era.

Abrupt stent thrombosis occurred at a rate of about 2% in the first year (essentially matching the natural risk of an untreated plaque). It continued at a rate of almost 1% per year.
year for several years. Hence, in contrast to POBA, there was a concern that mechanical plaque sealing with a stent never achieved a net outcome benefit in terms of preventing myocardial infarction. Of course, stents were welcome and unrivaled as a possible bailout measure in plaque sealing with POBA (Figure 3).

When drug-eluting stents (DES) were introduced, enthusiasm over having almost completely eliminated the risk of restenosis led to overzealous championing of mechanical plaque sealing by DES as the preferred treatment in intermediate lesions.9,10

The DEFER (DEFERtal of percutaneous coronary intervention)11 and FAME (Fractional flow reserve versus Angiography for Multivessel Evaluation)12 trials highlighted the importance of reserving stenting for lesions with an abnormal fractional flow reserve (FFR). Yet, only the bare metal stents used in DEFER, but not the DES used in FAME, proved inferior to deferred or omitted interventional treatment.13 It emerged from FAME-II14 (but was not discussed by the authors) that the registry patients from whom mechanical plaque sealing was withheld had the same percentage of angina at 1 year as patients with significant lesions receiving only medical treatment. This percentage hovered around 15% compared with only 3% in patients with coronary angioplasty. These patients were obviously at low risk for clinical events and few such events could have been prevented by stenting. Yet, the omission of stenting deprived them of possible clinical improvement.

**CONCLUSIONS AND OUTLOOK**

Weighing up lesion significance inevitably involves a multiplicity of parameters. A patient with a typical history and borderline lesion is likely to have the lesion viewed as significant and subsequently be offered dilation. This makes sense since the risk of complications or of more significant restenosis than at presentation is small with modern equipment and DES.

Typically, however, such a procedure is labeled as clinically indicated coronary stenting, and not as mechanical plaque sealing. FFR is not determined in such situations or else a normal FFR is ignored or perhaps repeated until an abnormal result is obtained.

Although it is possible to try and find out whether the lesion of interest is stable or a TCFA (Table I), the cost and risk involved appear unwarranted. The techniques used provide a snapshot assessment and cannot really predict what the lesion will look like days, weeks, months, or years down the line. In addition, most involve mechanical passage through the lesion, which may destabilize the situation and
make a stable plaque unstable. FFR itself provides a purely physiological assessment and only indirect evidence of instability (FFR tends to be lower in unstable lesions). Focusing on FFR misses the point: dilating FFR-positive lesions means only treating angina, but not fully exploiting the potential of preventing myocardial infarction. We need to ask ourselves which matters most: relieving angina or preventing infarction?

We should not fall victim to the oft-cited studies showing that most infarctions stem from nonsignificant lesions. We should keep in mind that the potential for infarction increases with the severity of stenosis (with the possible exception of chronically occluded lesions or lesions that were subtotal for many years, both fostering collateralization). The only reason that most infarctions are caused by non-significant lesions is that such lesions greatly outnumber the individually more dangerous and significant lesions.

Although studies such as a trial on exercise, COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation), FAME, MAS-II (Medicine, Angioplasty, or Surgery Study), and BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes) temporarily succeeded in dampening the enthusiasm of referring physicians and even interventional cardiologists for stenting mild lesions on sight, the transient dip they produced in the number of such procedures has all but recovered, to patients’ greater benefit. It is not surprising that after a mere couple of years patients with an untreated mild lesion should still be as well off as their treated peers, if we view them simply in terms of being alive and stable. However, their outlook is clearly bleaker than that of their stented counterparts. Conservatively managed patients still have the treatment (or worse, a spontaneous event) ahead of them in contrast to their already treated peers whose plaques are sealed.

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What are the pharmacological approaches to treat the vulnerable plaque?

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Pharmacological approaches can be divided into therapies that aim to reduce events by modifying key risk factors systemically and novel therapies to target vulnerable plaques and reduce the propensity for future rupture and complications. Despite the current standards of treatment for secondary prevention, further plaque ruptures occur and in about half of the cases, these are at de novo sites that are not involved in the index presentation. New targeted therapies are needed to prevent such plaque rupture-related events and their complications, to achieve high local concentrations of drugs or genetic manipulations in vulnerable plaques, and to optimize the balance between systemic hazard and local benefit. New technology, including the use of microspheres with surface ligands, already has the potential to deliver drugs or gene modifying therapy to vulnerable plaques.

Vulnerable plaques were initially characterized based upon the histopathological characteristics of disrupted plaques associated with myocardial infarction (MI) and were identified postmortem. Both conventional and more novel approaches to the identification of vulnerable plaques are set out in response to the question “how can the vulnerable plaque be identified (page 29)?”

These in vivo approaches are critical in order to identify the characteristics associated with the predisposition to rupture or erosion and to estimate the likelihood that such characteristics are associated with future plaque disruption. Although a “culprit lesion” can usually be identified with invasive or noninvasive imaging techniques, which include angiography, intravascular ultrasound, optical coherence tomography, virtual histology, intravascular ultrasound, near infrared spectroscopy, high resolution coherence tomography, and positron emission tomography (PET). The critical issue for the patient in the context of acute coronary syndrome (ACS) is the identification of vulnerable plaque(s) prior to plaque rupture and thrombotic complications. This is far more challenging than identifying the culprit lesion in the context of a disrupted plaque and ST elevation or non-ST elevation MI. Both the sensitivity and the specificity of the detection methods are important if a treatment regimen is focused on specific plaque characteristics. This article will consider treatments aimed at key features of the vulnerable plaque (lipid composition, inflammation, and fibrosis) and treatments that are designed to abrogate the complications of plaque disruption (thrombosis, occlusion). Substantial evidence indicates that vulnerable plaques do not occur in isolation and such patients exhibit systemic markers of risk; therefore, approaches are also required to treat the “vulnerable patient.”

**SELECTED ABBREVIATIONS AND ACRONYMIES**

<table>
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<th>ACN</th>
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<td>HO-1</td>
<td>heme oxygenase 1</td>
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<td>PCI</td>
<td>primary percutaneous coronary intervention</td>
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<td>STEMI</td>
<td>ST segment elevation myocardial infarction</td>
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Postmortem studies, invasive angiographic and ultrasound imaging, PET imaging, and biomarker studies have all identified evidence of multiple vulnerable plaques at the time of clinical presentation with ACS (Figure 1). In addition, intravascular ultrasound studies have revealed coincidental ruptures of nonculprit lesions at the time of ACS presentation (in 15% to 70% of cases). However, such plaques have also been identified in the context of apparently “stable” angina and in patients sustaining noncardiac deaths. Although novel techniques, now under development, have the potential to identify individual vulnerable plaques and target them with specific therapies, most of the development of imaging techniques are not in clinical application and hence current treatment strategies are mainly aimed at “vulnerable patients.” Novel approaches to the treatment of individual plaques will be discussed in the final section.

**TREating DISRuPTED VULNERABLE PLAQUES IN THE CONTEXT OF ACS**

Clear evidence supports the use of interventional techniques to restore vascular patency with primary percutaneous coronary intervention (pPCI), and if pPCI is unavailable, with fibrinolysis. The approaches are combined with platelet inhibition and anticoagulation to reduce the risk of thrombotic readmission. Dual antiplatelet therapy with aspirin and clopidogrel is superior to aspirin alone, and ticagrelor and prasugrel have both been demonstrated to be superior to clopidogrel. The potential for combining low doses of novel anticoagulants with antiplatelet therapy to reduce cardiac complications and mortality has been demonstrated, but each of the more potent antithrombotic regimens also increase the risk of bleeding. Inhibition of the thrombin receptor on platelets (PAR1) showed promise, but the risk-benefit balance for systemic administration may not be not favorable.

For NSTEMI, the results of interventional strategy trials were conflicting, but the combined analysis of all the trials of a systematic interventional strategy of angiography followed by revascularization vs a selective strategy based upon ischemia and symptoms demonstrated clear superiority for the interventional strategy, both in the short-term and over 5-years. The greatest absolute benefit was demonstrated in those characterized as being at the highest risk. Such treatments need to be applied in addition to secondary pharmacological therapies and lifestyle modifications (especially smoking cessation) with the aim of reducing the frequency and complications of subsequent plaque ruptures.

**PHARMACOLOGICAL APPROACHES AIMED AT MODIFYING FUTURE PLACe DISRUPTION**

**Lipid modification**

Substantial evidence supports the role of a pharmacological reduction in low-density lipoprotein cholesterol (LDL-C) as a means of reducing...
subsequent cardiovascular events including plaque rupture–related events. During atherogenesis there is a subendothelial accumulation of apolipoprotein (apo) B-containing lipoproteins, partially oxidized LDL, and remnant lipoproteins that is associated with an altered expression of adhesion molecules on endothelial cells over the plaque. Abbreviation: TCFA, thin-cap fibroatheroma.

The 2010 Cholesterol Treatment Trialists’ collaboration clearly demonstrated consistent reductions in cardiovascular events for each mmol/L reduction in LDL-C, independent of the baseline LDL-C. Both statin treatment trials and genetic studies based upon Mendelian randomization demonstrate that the longer
the individual is exposed to lower LDL-C the greater the hazard reduction. Further, the PROVE-IT trial (PRavastatin or AtorVastatin Evaluation and Infection Therapy) clearly showed that more intensive lipid lowering (atorvastatin 80 mg) reduced death or major cardiovascular events compared with moderate lipid lowering (pravastatin 40 mg). Overall, the Cholesterol Treatment Trialists’ collaboration has demonstrated, with very robust evidence, that more aggressive statin treatment reduces cardiovascular events (hazard ratio [HR], 0.72; 95% confidence interval [CI], 0.66-0.78) and that statins are more effective than the control (HR, 0.79; 95% CI, 0.77-0.81). More detailed investigations on the impact of intensive lipid lowering on atherosclerotic disease burden has been undertaken using coronary plaque volume assessment with intravascular ultrasound. These investigations have demonstrated that a more aggressive statin treatment resulted in greater reductions in LDL-C and CRP. There was no significant change in plaque volume with atorvastatin, however, pravastatin led to plaque progression as demonstrated by an increase in plaque volume. Overall, the findings suggest that changes in plaque behavior occur with more intensive lipid lowering or statin treatment vs control over and above any structural changes in plaque volume. The findings support the concept that lowering LDL-C alters the behavior of potentially vulnerable plaques and reduces the susceptibility to subsequent plaque rupture.

The JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) investigated rosuvastatin vs placebo among individuals with low LDL-C, but elevated high sensitivity CRP. This trial showed a reduction in MI, stroke, and cardiovascular death, and a reduction in arterial revascularization or hospitalization for unstable angina (HR =0.53) in patients treated with rosuvastatin. Thus, even among those patients with apparently optimal levels of circulating LDL-C, rosuvastatin treatment can reduce incident MI, stroke, and cardiovascular death.13

### Lipid modification: novel therapies

Epidemiological studies among populations of hunter-gatherers and studies in healthy neonates demonstrate that populations with low levels of LDL-C (<1.8 mmol/L) have very low risks of atherosclerosis. Loss of function mutations linked to the convertase subtilisin/kexin type 9 (PCSK9) proprotein are associated with reduced plasma levels of LDL-C and protection from ischemic heart disease. Conversely, PCSK9 gain of function mutations, although rare, cause hypercholesterolemia and premature ischemic heart disease. Novel approaches to lower LDL-C can be achieved with an injectable inhibitor of PCSK9 with 50% to 70% reductions in LDL-C compared with baseline. These studies open the possibility of profound lipid modification in patients with heterozygous or homozygous familial hyperlipidemia and those with resistant polycythemias. Also in such patients there is the potential to modify the behavior of the vulnerable plaque.

Cholesterol ester transfer protein (CETP) inhibitors have shown potential to increase high density lipoprotein (HDL) cholesterol, but efficacy studies have not yet been associated with significant improvements in outcome because of adverse effects including minor elevation of blood pressure with some agents. HDL mimetics and cholesterol absorption inhibitors are also being studied.

Heme oxygenase 1 (HO-1) and its metabolites have been implicated in the cytoprotective defense against oxidative injury in atherogenesis and in experimental studies. Induction of HO-1 reverses plaque progression from a vulnerable plaque appearance to a more stable phenotype. However, this work has been performed in mice and there are challenges when extrapolating to humans. Nevertheless, atherectomy specimens from patients with carotid artery disease were assessed for HO-1 expression and this correlated closely with vulnerable human atheromatous plaque features, including macrophage and lipid accumulation, which is inversely correlated with intraplaque vascular smooth muscle cells and collagen deposition. Further, HO-1 expression is associated with plaque stabilization factors including matrix metalloproteinase (MMP)-9, interleukin (IL)-8, and IL-6. This work opens the possibility for pharmacological enhancement of HO-1 levels to prevent lesion progression and rupture, and to reduce the risk of subsequent coronary events.

Lipid lowering produces early changes in endothelial function, but the impact on thrombotic events is more delayed and changes in the plaque lipid pool are modest and take months to be measurable. Studies of LDL reduction in animals demonstrate that changes occur in the structure of plaques with reduction in macrophage numbers, reduced MMP-1 expression, increased interstitial collagen, and consequently, a more stable plaque structure.

Long-chain omega-3 fatty acids, which occur naturally in fish oils and various components including eicosapentaenoic acid, have shown beneficial effects in experimental- and small-scale clinical studies, but
Potential to directly modify inflammation in the plaque

The development of atheromatous plaques involves a maladaptive inflammatory response to lipoproteins in the subendothelial space. A failure to resolve inflammation promotes the development of vulnerable plaques, and a critical balance exists between inflammation and repair. The clearance of apoptotic cells is influenced by a cascade of anti-inflammatory cytokines, lipoxygenase-derived lipids, and transcription factors.

Alternatively activated macrophages (M2) suppress inflammation and engulf cellular debris. M2 macrophages are converted from classically activated macrophages (M1) under the influence of cytokines including IL-10 (from T2 helper cells). Peroxisome proliferator-activated receptor gamma (PPARγ) is induced by IL-4 and suppresses inflammation in macrophages. A series of growth factors (in particular, granulocyte-macrophage colony stimulating factor [GM-CSF], tumor necrosis factor [TNF], IL-4, IL-6, IL-10, transforming growth factor β [TGFβ]) are potentially amenable to manipulation as they promote differentiation and maturation of inflammatory myeloid cells as well as initiation of inflammation resolution (Figure 2, 3).

IL-10 has a key role in suppressing proinflammatory cytokine production and secretion. IL-10 also activates the signal transducer and activator of transcription 3 (STAT3) and attenuates stress induces apoptosis in macrophages. TGFβ acts by suppressing apoptosis, inducing collagen formation, and inducing formation of the “fibrous cap” over the plaque. Liver X receptor (LXR) activation stimulates cholesterol efflux from lipid laden macrophages, promotes plaque regression in murine models, and also inhibits MMP-9, which is potentially pivotal for plaque disruption. These pathways are critical for successful resolution of inflammation and formation of the protective fibrous cap. Thus, this complex balance of inflammation and repair has the potential for pharmacological manipulation toward inflammation resolution if suitable agents can be delivered in sufficient concentrations into vulnerable plaques. This “targeted” approach has much in common with the localization of chemotherapeutic agents in tumors. Perhaps cardiology has much to learn from the novel approaches of molecular targeting in oncology?

In contrast, several anti-inflammatory approaches in cardiology have been more broadly based and not specifically targeted toward the plaque. Systemic administration of corticosteroids has been attempted and a meta-analysis of 11 trials (2646 patients) suggested a 26% decrease in mortality in treatment following acute MI, but the decrease was no longer significant when the analysis was limited to randomized controlled trials (odds ratio [OR], 0.95, 95% CI, 0.72-1.26). There are hazards of systemic administration of steroids after acute MI, including impaired infarct healing and the potential for myocardial rupture.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for noncardiac indications, but all of the agents with the exception of aspirin increase the risk of MI. Studies in arthritis suggest that administration of methotrexate may be associated with reduced cardiac events. In addition, a retrospective cross-sectional study of 1288 patients with gout demonstrated that colchicine use was associated with a lower incidence of MI (1.2% vs 1.6%, \(P=0.03\)) than nonuse. In the first trial of colchicine as an anti-inflammatory therapy in coronary disease, Nidorf and colleagues used a prospective randomized observational end point design of 332 patients who were assigned to colchicine 0.5 mg/day or no colchicine. The findings suggest a reduction in ACS events with colchicine treatment (4.6% vs 13.6%). These results are promising and raise the possibility that colchicine may modify the behavior of vulnerable plaques, but they require prospective validation in larger trials. In the context of stable coronary artery disease, the same authors have reported marked reductions in CRP levels with colchicine treatment.

Ongoing trials are investigating other anti-inflammatory agents. For example, the CANTOS study (Canakinumab Anti-Inflammatory-Thrombosis Outcomes Study) is using canakinumab, a monoclonal antibody against IL-1β, in stable patients following MI and with a long-term follow-up. A separate study will determine whether low doses of methotrexate will reduce cardiovascular events following MI, among patients with elevated CRP and type-2 diabetes. Also, a trial has investigated the effect of an IL-1 receptor antagonist on markers of inflammation in NSTEMI using an agent currently available for the treatment of arthritis.

In summary, despite the complexity of the inflammatory processes in vulnerable plaques, several of the pathways are mediated via the same dietary supplementation of fish oils in large-scale studies of patients (12,500 patients) with manifest vascular disease has not shown evidence of benefit. The outcomes (death, MI, stroke, cardiovascular hospitalization) for placebo or supplementation with omega-3 fatty acids, over five years, were virtually superimposable.
effector molecules and these are feasible targets for currently available drugs. This explains part of the current benefits, but novel therapies are likely to require specific targeting to vulnerable atheromatous plaques in order to achieve effective local concentrations.

**Modification of sheer stress**

Thin-cap fibroatheromas (TCFAs) are associated with the propensity to rupture based upon a large necrotic core, a high content of leukocytes, and a thin fibrous cap. Changes in sheer stress impact on the arterial wall can upregulate inflammatory signaling in endothelial cells and leukocytes. Consequently, there is modulation of microRNAs, promotion of inflammation, and monocyte recruitment. 34

Fast-flowing blood in straight vessels generates high sheer stress patterns, but low sheer stress occurs in the inner curvature of vessels and distal to stenosis. There is substantial experimental evidence to link low sheer stress to disruption of vulnerable plaques. Similarly, oscillatory sheer stress involves changes in the magnitude of sheer stress and the direction of blood flow, and these patterns are associated with vulnerable plaques. 35 The impact of β-blockers following MI may, in part, be related to changes in wall stress with similar potential benefits associated with lowering blood pressure.

Rupture of TCFAs may be associated with the impact of sheer stress on microcalcification in plaque and within cells. Such microcalcification (=10 μm diameter) is below the resolution of current ultrasound imaging devices, but could be assessed using optical coherence tomography or micro-computed tomography.

**ACE inhibitors**

Angiotensin-converting enzyme (ACE) inhibitors modify endothelial dysfunction and free oxygen radical production caused by angiotensin, decrease macrophage activity, and inhibit smooth muscle cell lipoxigenase; therefore, they have the potential to improve plaque stability. In animal studies, reduced atherosclerosis develops in the presence of ACE inhibitors even in nonhypertensive models and these studies demonstrated improved endothelial function and coronary flow. The benefits of ACE inhibitors in large-scale studies have been postulated to be due, in part, to plaque stabilization. There is an upregulation of collagen type III synthesis and antiatherogenic effects as exhibited by a reduction in the rate of carotid intima media thickening. 36,37

Some antioxidants may contribute to plaque stabilization by inhibiting LDL-C oxidation and altering vascular reactivity. Diets rich in antioxidants, such as the Mediterranean diet, are associated with reduced coronary events, but large-scale trials of omega-3 essential fatty acids have been disappointing. 18

**Potential to focus treatment on individual vulnerable plaques**

The conventional pharmacological approaches to the prevention of cardiovascular adverse events has involved systemic administration of drugs to populations characterized by their clinical phenotype (eg, hypertension, hyperlipidemia, post-MI). However, the potential already exists to alter the risk-benefit balance by focusing novel treatments on patients at higher risk for events, but lower risk of harm. These “personalized” approaches can involve the use of risk scoring tools (Euroscore, CHADS2-VASC, GRACE, TIMI) and there is the potential to improve the risk-treatment balance with genetic profiling (eg, using markers of altered warfarin or clopidogrel metabolism). Novel future therapies aim to achieve high local
concentrations of pharmacologically or biologically active agents at the sites where there is the greatest potential for benefit. An established example is the use of drug eluting stents to limit restenosis, rather than systemic administration of cell cycle inhibitors.

However, the next “quantum step” in focused treatment of vulnerable plaques will involve approaches analogous to those already adopted in oncology. Chemotherapeutic agents can achieve high local concentrations by taking advantage of the change in expression of surface receptors on atheromatous plaques, the exposure of plaque contents, and the subendothelial matrix in disrupted plaques (Figure 4). By linking an active molecule to a monoclonal antibody, or other ligand, high local concentrations can be achieved. Novel approaches involve the use of synthetic microspheres to deliver the biologically active (potential gene therapy) or chemically active agents (drugs) to the site of plaque disruption.38-41 Specific ligands can be attached to the surface of microspheres using linking molecules. The specific ligand allows the microspheres to concentrate at the site of plaque disruption. Once attached to the site of interest, the microspheres can be disrupted using a burst of ultrasound energy, releasing the biologically active content in high local concentrations. Early studies demonstrate that this concept is feasible and no longer science fiction.38-41 The time has come for bold steps in order to change the behavior of vulnerable plaques by exploiting our understanding of the key inflammatory and repair pathways within vulnerable plaques and novel technological approaches to deliver biologically active agents to specific sites where they can modify the balance toward repair.

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Therapeutic approaches to the vulnerable plaque


The Vulnerable Plaque

Summaries of Ten Seminal Papers

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1. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina

2. Plaque rupture and sudden death related to exertion in men with coronary artery disease
   A. P. Burke and others. JAMA. 1999

3. Perturbation of the T cell repertoire in patients with unstable angina
   G. Liuzzo and others. Circulation. 1999

4. Widespread coronary inflammation in unstable angina

5. Is there a vulnerable plaque?
   A. Maseri and V. Fuster. Circulation. 2003

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Selection of seminal papers by Filippo Crea, MD, FESC, FACC
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Highlights of the years by Sherri Smith, PhD
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Unstable angina pathogenesis is poorly understood. A role for inflammation has been implicated due to elevated levels of acute phase proteins such as C-reactive protein (CRP), however, at that time, studies analyzing the prognostic importance of this elevation had not been conducted. Therefore, Liuzzo et al utilized new highly sensitive immunoassays to detect two acute phase proteins, CRP and serum amyloid A protein (SAA), as well as troponin T, a specific myocardial necrosis marker. The authors measured CRP, SAA, and troponin T in plasma samples from: (i) 32 patients with chronic stable angina; (ii) 31 patients with severe unstable angina; and (iii) 29 patients with acute myocardial infarction.

At the time of hospital admission, troponin T levels were normal in all patients, while CRP and SAA levels were >0.3 mg per deciliter (exceeding the 90th percentile of normal distribution) in 4 patients with stable angina (13%), in 20 patients with unstable angina (65%), and in 22 patients with acute myocardial infarction (76%). The 20 patients with unstable angina and increased levels of CRP had more ischemic episodes in the hospital than those with normal CRP levels (mean ±SD number of episodes per patient, 4.8±2.5 vs 1.8±2.4, respectively; \( P=0.004 \)). Subsequently, 5 patients had a myocardial infarction, 2 patients died, and 12 patients required an immediate coronary revascularization. In contrast, no deaths or myocardial infarction occurred among the 11 patients with normal levels of CRP and SAA. Similar results were observed among patients admitted with acute myocardial infarction.

Myocardial necrosis has been shown to be a potential stimulus of acute phase reactants. This study revealed that troponin T levels remained within the normal range, whereas both CRP and SAA were increased, which indicates that their response is not induced by myocardial necrosis. Additionally, SAA is predicted to be a better choice for routine testing because the SAA assays are more sensitive than the assays for CRP.

Although the number of patients was small, this study was the first to demonstrate the key role of inflammation in acute coronary syndromes. Therefore, this study opened the way to transition from immune system biology to clinical practice by using inflammatory biomarkers to predict cardiovascular risk, monitor treatments, and guide therapy.
Exercise has been well established to reduce the risk of coronary heart disease and death. Contrarily, exercise exertion has been reported to acutely increase the risk of sudden coronary death, but the underlying mechanisms are unclear. At this time, the working hypothesis was that acute exertion may predispose persons to sudden coronary events by triggering the rupture of vulnerable coronary artery plaques. The aim of this study was to determine the frequency of plaque rupture in sudden coronary deaths related to exertion compared with sudden coronary deaths not related to exertion.

Burke et al conducted their research using an autopsy survey. Coronary arteries were perfusion fixed and segments with more than 50% luminal narrowing were histologically examined. Ruptured plaques were defined as intraplaque hemorrhages with a disruption of the fibrous cap and luminal thrombus. The study analyzed a total of 141 men with severe coronary artery disease who died suddenly, including 116 whose deaths occurred at rest, and 25 who died during strenuous activity or heavy emotional stress.

The frequency and morphology of plaque rupture was compared between men dying at rest vs those dying during exertion. In addition to acute exertion, independent association of risk factors with plaque rupture (eg, total cholesterol, high-density lipoprotein cholesterol, glycylated hemoglobin, cigarette smoking) were determined.

The number of vulnerable plaques (mean±SD) in the coronary arteries of men in the exertion-related death group was 1.6±1.5 vs 0.9±1.2 (P=0.03) in the at-rest group. The culprit for men dying during exertion was plaque rupture in 68% vs 23% for men who died at rest (P<0.001). Hemorrhage into the plaque occurred in 72% of men in the exertion-related death group and 41% of men in the rest-related death group (P=0.007). In multivariate analysis, both exertion (P=0.002) and the ratio of total cholesterol to high-density lipoprotein cholesterol (P=0.002) were associated with plaque rupture, independent of age and other cardiac risk factors.

This study demonstrated that acute exertion is an additional and independent risk factor for plaque rupture in men, most likely by disruption of a vulnerable plaque. Of note, stress-related plaque fissures exhibited a thinner cap as compared with plaque fissures occurring at rest, suggesting greater susceptibility to biomechanical forces. Therefore, this study suggests that mechanical, rather than inflammatory, rupture of a thin cap, which is triggered by a systemic sympathetic surge, can cause coronary instability.

Bertrand Piccard and Brian Jones become the first to circumnavigate the world in a hot air balloon without stopping—taking 19 days, 21 hours, and 55 minutes; Boris Yeltsin resigns as President of Russia and is replaced by Vladimir Putin; and Michel Petrucciani, an accomplished French jazz pianist, dies at age 36 from a pulmonary infection.
Accumulation of inflammatory cells (e.g., activation of monocytes in coronary lesions) and subsequent activation of inflammatory pathways in patients with unstable angina (UA) are hypothesized to be involved in the development of acute coronary syndromes. Monocytes are constitutively activated in UA, resulting in the production of IL-6 and upregulation of acute phase proteins (e.g., C-reactive protein and serum amyloid A protein). The mechanisms mediating monocyte activation are unknown, but may be due to IFN-γ that is released from activated T lymphocytes.

To explore whether the production of IFN-γ, a potent monocyte activator, is altered in UA, the authors compared the cytokine production (IFN-γ, IL-2, and IL-4) from circulating CD4+ and CD8+ T cells in patients with either UA or stable angina (SA).

Peripheral blood lymphocytes were collected at the time of hospitalization and again after 2 and 12 weeks. Cytokine-producing CD4+ and CD8+ T cells were quantified by 3-color flow cytometry after stimulation with phorbol myristate acetate and ionomycin. UA was associated with an increased number of IFN-γ+CD4+ and IFN-γ+CD8+ T cells, whereas, patients with SA had higher frequencies of IL-2+CD4+ and IL-4+CD4+ T cells. The frequency of the IFN-γ+ T-cell population in UA declined 1 to 2 weeks after discharge from the hospital before returning to their high levels after 3 months. Increased production of IFN-γ in UA could be attributed to the expansion of CD4+CD28null T cells, an unusual subset of T cells that occurred with the same frequency as IFN-γ secreting T cells. Previously, the authors demonstrated that CD4+CD28null T cells represented pro-inflammatory cells with a high potential for causing tissue damage.

Immune cells infiltrating coronary lesions have been suspected to contribute to plaque instability. This investigation suggests that global immune system alterations are of functional importance for plaque inflammation. The authors have concluded that patients with UA are characterized by a perturbation in functional T cell responses and cytokine production with a bias toward IFN-γ production, suggesting that monocyte activation and acute phase responses are consequences of T-cell activation. IFN-γ is produced by CD4+CD28null T cells, which are expanded in UA and low in both SA and control patients. The emergence of CD4+CD28null T cells may result from persistent antigenic stimulation.

This is the first study showing that a profound perturbation in adaptive immunity plays a key role in patients with acute coronary syndromes.

Nikolai William Alexander Frederik, Prince of Denmark and Count of Monpezat, is born and is 3rd in line for the throne after his uncle and father; 33-year-old Billy Mitchell achieves a perfect score in the video game *Pac-Man*—the first person to do so since the games’ inception almost 20 years before; and the largest bacterium, *Thiomargarita namibiensis*, is discovered in ocean sediments of the Namibia continental shelf and is 0.1 to 0.3 mm in diameter—almost 600 times larger than the average bacterium.
In unstable angina, activated leukocytes may be found in peripheral and coronary-sinus blood, but it is unclear whether they are selectively activated in the vascular bed of the culprit stenosis. In this study, neutrophil myeloperoxidase content was measured in both the cardiac and femoral circulations in the following five groups of patients: (i) unstable angina and stenosis in the left anterior descending coronary artery (24 patients); (ii) unstable angina and stenosis in the right coronary artery (9 patients); (iii) chronic stable angina (13 patients); (iv) variant angina and recurrent ischemia (13 patients); and (v) control (6 patients). Blood samples were taken from the aorta, the femoral vein, and the great cardiac vein, which selectively drains blood from the left, but not the right coronary artery.

The neutrophil myeloperoxidase content of aortic blood was similar in both groups of patients with unstable angina (~3.9 and -5.5, respectively, with negative values representing enzyme depletion due to neutrophil activation) and was significantly lower than that observed in the other three groups ($P<0.05$). Independent of the site of stenosis, the neutrophil myeloperoxidase content in blood from the great cardiac vein was significantly decreased in both groups of patients with unstable angina (~6.4 in those with a left coronary lesion and ~6.6 in those with a right coronary lesion), but not in patients with stable angina and multiple stenoses, patients with variant angina and recurrent ischemia, or control patients. There was also a significant transcardiac reduction in myeloperoxidase content in both groups with unstable angina.

The widespread neutrophil activation across the coronary vascular bed in patients with unstable angina, regardless of the location of the culprit stenosis, challenges the concept of the involvement of a single vulnerable plaque in unstable coronary syndromes.

This study has a number of important implications. First, it suggests that patients with an increased risk of acute coronary events are likely to have many vulnerable lesions throughout the coronary tree. As a consequence, current focal therapies for coronary artery disease (eg, angioplasty, stent implantation, etc) are not likely to prove entirely useful for reducing the incidence of clinical events in such high-risk patients, supporting the notion that plaque vulnerability may be best modulated by a systemic therapeutic approach.

Widespread coronary inflammation in unstable angina

A. Buffon, L. M. Biasucci, G. Liuzzo, G. D’Onofrio, F. Crea, A. Maseri


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2002

French President Jacque Chirac escapes assassination by would-be assassin Maxime Brunerie during Bastille Day celebrations; Robert William Pickton, a pig farmer and the most prolific serial killer in Canadian history, is arrested and is charged with fifteen counts of first-degree murder—of an eventual twenty-seven; and Alfred Henry Heineken, chairman and CEO of Heineken, dies at age 78 as one of the richest persons in the Netherlands with a net worth of 9.5 billion Dutch guilders.
Is there a vulnerable plaque?

A. Maseri, V. Fuster

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Maseri and Fuster have contributed enormously over the years to our knowledge of the mechanisms of acute coronary syndromes (ACS). In this publication, the authors have reviewed the current knowledge in the field. Vulnerable plaque, which is characterized by positive remodeling, a large lipid core, and a thin fibrous cap, has been implicated in the development of unstable angina, myocardial infarction, and sudden cardiac death. The authors convincingly demonstrated that this popular concept is overly simplistic and inadequate to explain the clinical spectrum of atherothrombotic events. For example, plaque inflammation may be present in patients with chronic stable angina, yet absent in some patients who present with an ACS. Plaque rupture may result in a stable, mural, and endoluminal thrombus and contribute to gradual and stepwise plaque growth. Alternatively, plaque rupture may precipitate catastrophic abrupt coronary occlusion.

As emphasized by the above-mentioned thought leaders, the concept of a vulnerable plaque as the final common pathway by which atherothrombotic events occur ignores the multiple and diverse triggers for acute coronary events as well as the important contributions of blood rheology and the coagulation cascade (high-risk blood). Therefore, several questions must be clarified regarding whether vulnerable or high-risk plaques may: (i) become unstable because of a structural or an inflammatory vulnerability; (ii) present simultaneously in multiple coronary arteries; (iii) remain vulnerable for weeks, months, or years; and (iv) be fibrotic, without a lipid-rich core and thin fibrous cap.

To specifically define the concept of vulnerable or high-risk plaques as potential precursors of unstable lesions that may trigger ACS, it is useful to consider the distinctive structural and functional features of the culprit unstable coronary plaques and the distinctive clinical presentation of ACS. The most obvious features that distinguish patients with ACS from patients with stable coronary artery disease include: (i) complex coronary stenosis; (ii) coronary plaque fissures; (iii) fresh thrombi; and (iv) plaque inflammation.

Collectively, the data reviewed suggests that the precursors of unstable plaques that trigger ACS are multiple and complex, both structurally and functionally. Moreover, in some cases, unstable or otherwise stable plaques may require thrombogenic high-risk blood to trigger ACS. Such potentially diverse origins of plaque vulnerability have relevant implications for their temporal evolution of vulnerability as well as for the selection of diagnostic and passivation strategies.

This review clearly exposes the limitations and inadequacies in the “one shoe” concept of vulnerable plaque that “fits all” aspects for pathogenesis of vascular atherothrombotic events and instead provides a more dynamically inclusive framework for the understanding of coronary instability.

Katharine Hepburn, an American actress who received four academy awards for best actress, dies at age 96; Prometea, the first cloned horse, is born by natural delivery from her cloning mother who carried her to a full-term pregnancy; and Arnold Schwarzenegger, an Austrian-American actor and former bodybuilding champion, is elected governor of California.
Cholesterol crystals are often observed in abundance within atheromatous plaques and at the sites of plaque disruptions; however, their role in plaque disruptions have not been determined. Standard histological studies are detrimental to the observation of cholesterol crystals because the solvents utilized during tissue processing dissolve the crystals, which makes it difficult to correctly analyze the role of cholesterol crystals in plaque disruption. Therefore, the authors used an alternative procedure of vacuum dehydration to visualize the full effect of the cholesterol crystals. This study investigated the hypothesis that cholesterol crystals can damage plaques and intima, thereby triggering plaque disruption.

Coronary arteries of patients who died from an acute coronary syndrome (n=19) or a nonacute coronary syndrome (n=12), and carotid plaques from patients with (n=51) or without (n=19) neurologic symptoms were studied. Tissues were prepared without using ethanol solvents that dissolve cholesterol crystals and were examined for cholesterol crystals that had perforated the intima using light microscopy and scanning electron microscopy (SEM). In addition, fresh unfixed carotid plaques were examined at 37°C using confocal microscopy. Using SEM, crystal content was scored from 0 to +3. SEM using vacuum dehydration had significantly higher crystal content and showed enhanced detection of cholesterol crystal perforations compared with SEM using ethanol dehydration (+2.5±0.53 vs +0.25±0.46; \(P<0.0003\)).

The presence of cholesterol crystals using SEM and confocal microscopy was similar, suggesting that cholesterol crystal perforation can occur in vivo at 37°C. All patients with acute coronary syndromes had perforating cholesterol crystals, but no crystals were present in patients without acute coronary syndromes (\(P=0.0001\)). For all plaques, cholesterol crystals were strongly associated with plaque disruption, thrombus, symptoms (\(P<0.0001\)), and plaque size (\(P<0.02\)). Crystal content was an independent predictor for thrombus, and there was a significant association between increasing cholesterol crystal content and increasing clinical symptoms. In conclusion, by avoiding ethanol in tissue preparation, cholesterol crystals perforating the intima were shown to be associated with plaque disruption. Crystal content was significantly associated with clinical events, suggesting that cholesterol crystallization may have a role in plaque disruption.

This study demonstrates, for the first time, that mechanical rupture of a thin plaque caused by local cholesterol crystallization is a potential mechanism of coronary instability. The clinical implication of the association between cholesterol crystals and plaque disruption may be to develop strategies to detect cholesterol crystals perforating the intima, followed by targeted pharmacological and/or mechanical interventions to prevent or reverse cholesterol crystallization with consequent plaque disruptions.
Systemic levels of myeloperoxidase, a hemeprotein stored in azurophilic granules and released upon neutrophil activation, can predict the prognosis in patients with acute coronary syndromes (ACS) and is considered to be a marker of plaque vulnerability. However, it is not known whether myeloperoxidase is associated with different coronary morphologies (ie, rupture or erosion of the culprit lesion) in patients with acute coronary syndrome. Therefore, the authors assessed whether systemic levels of myeloperoxidase in ACS patients reflected the presence of erosion or rupture of the culprit plaque.

To this end, the assessment was conducted in vivo using optical coherence tomography. Furthermore, the authors investigated whether intraluminal thrombi overlying eroded or ruptured plaques in postmortem coronary specimens had differing amounts of myeloperoxidase-positive cells in patients who died suddenly.

In this study, 25 consecutive patients (aged 67±11 years; 15 men [60%]) were enrolled. Thirteen patients (52%) presented with non-ST segment elevation ACS and, of these, 4 (16%) had unstable angina and 9 (36%) had non-ST segment elevation myocardial infarction (NSTEMI). Twelve patients (48%) presented with ST-segment elevation myocardial infarction (STEMI) and, of these, 5 (20%) had an acute STEMI and 7 (28%) had a subacute or recent STEMI.

Optical coherence tomography classified the culprit lesion as ruptured in 72% or eroded in 28% of patients. Baseline systemic serum myeloperoxidase levels were significantly higher ($P=0.001$) in patients with an eroded plaque (median, 2500 ng/mL; 25th to 75th percentile, 1415 to 2920) than in those with a ruptured plaque (median, 707 ng/mL; 25th to 75th percentile, 312 to 943), whereas C-reactive protein levels were not significantly different (median, 11.3 mg/L; 25th to 75th percentile, 1.3 to 28.5 vs median, 3.9 mg/L; 25th to 75th percentile, 1.3 to 17.8; $P=0.76$). In addition, the density of myeloperoxidase-positive cells within thrombi overlying plaques in postmortem coronary specimens retrieved from sudden coronary death victims was significantly higher in lesions with erosion (n=11) than ruptures (n=11) (median, 1584; 25th to 75th percentile, 1088 to 2135 cells/mm$^2$ vs median, 579, 25th to 75th percentile, 442 to 760 cells/mm$^2$; $P=0.0012$).

In conclusion, systemic myeloperoxidase levels were significantly elevated in acute coronary syndrome patients presenting with an eroded plaque compared with patients presenting with a ruptured plaque. Consistently, in postmortem coronary specimens, luminal thrombi superimposed on eroded plaques contained a higher density of myeloperoxidase-positive cells than thrombi superimposed on ruptured plaques. This study supports the notion that elevations in selective inflammatory biomarkers reflect specific acute complications for coronary atherothrombosis.
Enhanced Rho-kinase activity in circulating neutrophils of patients with vasospastic angina: a possible biomarker for diagnosis and disease activity assessment


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Rho-kinase pathway activation plays a central role in the molecular mechanism of coronary vasospasm in animal models and patients with vasospastic angina (VSA). Recently, it has been reported that Rho-kinase activity in circulating leukocytes is associated with various diseases. The aim of this study was to examine whether Rho-kinase activity is systemically enhanced in patients with VSA and, if so, whether a noninvasive diagnostic method could be developed to improve practice.

Fifty-three consecutive patients with chest pain who underwent acetylcholine provocation testing for coronary spasms were examined. Patients were divided into 2 groups depending on their response to the test: VSA (n=33) and non-VSA (n=20) groups. Venous blood samples were collected to measure Rho-kinase activity in circulating neutrophils, which was determined by the extent of phosphorylation of the myosin-binding subunit (MBS), a substrate of Rho-kinase.

Rho-kinase activity was significantly higher in the VSA group vs the non-VSA group (phosphorylated MBS/total MBS ratio, 1.33±0.37 vs 0.95±0.22, \( P<0.001 \)). In the VSA group, no correlation was observed between Rho-kinase activity and high-sensitivity C-reactive protein, smoking, or an accumulated number of coronary risk factors. After a 3-month medical treatment, Rho-kinase activity in the VSA group significantly decreased to 1.08±0.31 (\( P<0.001 \)), which was correlated with a significant reduction in symptom severity. After a receiver-operating characteristic curve analysis, a phosphorylated MBS ratio of 1.18 was identified as the best cutoff level to predict the diagnosis of VSA.

The novel data provided by Kikuchi et al has important pathogenetic and clinical implications and, at the same time, raises some stimulating new questions. The demonstration that neutrophil Rho-kinase activity is increased in VSA patients lends further support to the notion that enhanced Rho-kinase activity in smooth muscle cells (SMCs) may contribute to the pathogenesis of coronary artery spasms. However, the demonstration of systemic enhancement of Rho-kinase activity fails to explain the observation that coronary artery spasms are usually localized to a specific coronary segment or branch, suggesting the presence of an unknown local factor that causes local SMC hyperactivity by further enhancement of SMC Rho-kinase activity and/or by involvement of other constrictor pathways.

This study also has clinical implications as it indicates that Rho-kinase activity in circulating neutrophils is enhanced in patients with VSA and may be a useful biomarker for diagnosis and disease activity assessment of the vasospastic disorder.

Grete Waitz, a Norwegian marathon runner who won 9 New York City marathons, dies at age 57 from cancer; elements 114 (flerovium) and 116 (livermorium) are officially added to the periodic table, becoming the heaviest elements added to date; and Susana Chávez, a Mexican poet and human rights activist, is murdered in her home town of Ciudad Juárez at the age of 36.
Summaries of Ten Seminal Papers - Crea

Imaging intraplaque inflammation in carotid atherosclerosis with $^{11}$C-PK11195 positron emission tomography/computed tomography


Eur Heart J. 2012;33:1902-1910

Gaemperli et al have conducted a proof of principle study to analyze the value of using the recently developed positron emission tomography (PET) tracer $^{11}$C-PK11195 to determine whether intraplaque inflammation could reliably be measured using combined PET and computed tomography angiography (CTA) imaging. $^{11}$C-PK11195 is a selective ligand for the translocator protein (TSPO), which is highly expressed in activated macrophages of the mononuclear phagocyte lineage. Recently, specific in vitro binding of $^{3}$H-PK11195 to macrophages has been demonstrated in human carotid endarterectomy samples and a pilot study in patients with large vessel vasculitis has shown that $^{11}$C-PK11195 PET/CTA can be used to assess vascular inflammation in vitro. Therefore, this study had two aims: (i) to assess $^{11}$C-PK11195 PET/CTA for measuring inflammation of carotid atherosclerotic plaques in vivo; and (ii) to determine if it can discriminate between recently symptomatic and asymptomatic lesions.

To this end, they recruited symptomatic (n=9) and asymptomatic (n=27) patients with carotid artery stenosis for $^{11}$C-PK11195 PET/CTA imaging. On CTA images, plaque composition was assessed by measuring CT attenuation of the carotid plaque. $^{11}$C-PK11195 uptake into carotid plaques was measured using target-to-background ratios (TB Rs). The study showed that patients with a recent cerebral ischemic event had ipsilateral plaques with lower CTA attenuation and higher $^{11}$C-PK11195 uptake. Both TB Rs and CT plaque attenuation had high negative predictive values (91% and 92%, respectively) for detecting symptomatic patients. However, the best positive predictive value (100%) was achieved when TB Rs and CT attenuation were combined. In those patients who underwent carotid endarterectomy (n=8), ultrathin contiguous sections were processed for TSPO and CD68 labeling, using immunohistochemical staining, $^{3}$H-PK11195 autoradiography, and confocal fluorescence microscopy. By immunohistochemistry and confocal fluorescence microscopy, CD68 and TSPO were shown to colocalize with $^{3}$H-PK11195 uptake at autoradiography. Of note, a significant correlation was found between the $^{11}$C-PK11195 TBR and the percentage of autoradiographic specific binding ($r=0.77, P=0.025$), thus confirming the feasibility of assessing plaque and vascular inflammation with combined PET/CTA.

This ’proof of principle’ study is important, as it shows the potential of this noninvasive multimodal approach for the integral assessment of vascular inflammation and plaque vulnerability in patients. Unfortunately, the study did not compare the diagnostic and prognostic roles of imaging vs systemic markers of inflammation, and this should be an avenue for future research.

A transit of Venus, one of the rarest predictable astronomical phenomena in which Venus moves between the Earth and the sun, occurs and the next transit will not occur for another 105 years; Wales wins the Six Nations Championship in rugby to claim their 11th Grand Slam title; and Le Louvre-Lens, a branch of the Musée du Louvre, designed by SANAA, opens in Lens, Pas-de-Calais, France
Immune effector mechanisms implicated in atherosclerosis: from mice to humans

P. Libby, A. H. Lichtman, G. K. Hansson

Immunity. 2013;54:1092-1104

Libby et al reviewed the contributions to the pivotal role inflammation plays in all phases of atherothrombosis. Traditionally, it was thought that atherosclerosis resulted from a passive buildup of cholesterol in the artery wall, but recent advances in science have shown that inflammation and immune effector mechanisms are potentially involved in atherosclerosis pathogenesis. Therefore, the authors discussed the current experimental and clinical knowledge of the pathogenesis of atherosclerosis from the perspective of immunology taken from elegant experiments conducted in mice and how these experiments and results link to human disease. Additionally, the authors discussed the idea that while host defense mechanisms are essential for our survival, they are a contributor to this chronic disease and also present new opportunities for its mitigation.

The authors clearly explained how both innate and adaptive immunity operate during atherogenesis and link many traditional risk factors to altered arterial functions. They discussed how cells of the adaptive immune system (eg, T cells, antigen-presenting dendritic cells, B cells) and the signaling pathways involved (eg, transforming growth factor β [TGFβ] signaling) exert both exacerbating and inhibitory influences on lesion development. They also discussed how the involvement of innate immune effectors (eg, mononuclear phagocytes, mast cells, platelets, granulocytes, and the complement cascade) respond to cholesterol buildup in the arteries.

IFN-γ producing CD4+ T helper cells (Th cells) are predominant in mouse lesions and enhance lesion development. In humans, IFN-γ and IFN-γ/interleukin (IL)-17 producing cells are frequently associated with atherosclerotic lesions. Macrophages are major inflammatory cells present in both human and mouse lesions and typically are classically activated (M1). High-sensitivity C-reactive protein (hsCRP) is only slightly present in mouse lesions, but high hsCRP is a risk factor for clinical disease in humans. Cytokine signaling pathways often exhibit both exacerbating and inhibitory influences on lesions. For example, TGFβ, an anti-inflammatory cytokine, can modulate atherogenesis in hypercholesterolemic mice by balancing inflammation and fibrosis in atherosclerotic plaques. In fact, eliminating TGFβ signaling to T cells has been shown to accelerate experimental atherosclerosis. These results have suggested that a subset of T cells, known as regulatory T cells (Treg), are involved in controlling effector T cells, which are important atheroprotective cells.

Additionally, the authors highlighted how inflammatory pathways (eg, foam cell formation, pattern recognition receptor activation, macrophage differentiation after cholesterol stimulation, inflammasome activation) have become targets in the quest for novel preventive and therapeutic strategies against cardiovascular disease.

Finally, the authors emphasized the fact that while many experiments have been conducted in mouse models, relatively little is known regarding inflammation and immunity in human atherogenesis. The main problems associated with immunological studies in humans are the genetic diversity of the populations as well as environmental influences that cannot be controlled as they can in laboratory mouse model experiments. Application of contemporary genetic tools may provide help to further understand the role of inflammation during atherogenesis. Most importantly, the understanding that inflammation and immunity is important in this disease should provide opportunities and directions for future research into the topic.

Charles Ray’s sculpture “Boy with Frog” is removed by the city of Venice from the Punta della Dogana; researchers discover four key cholesterol genes in baboons, which may potentially lead to the development of new drugs to treat heart disease in humans; and Taiho Koki, the 48th sumo wrestler to become a yokozuna and winner of a record 32 tournaments, dies at 72.
The Vulnerable Plaque

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Is there a vulnerable plaque?

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