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—— Therapeutic Targets for the Treatment of Atherothrombosis in the New Millennium – Clinical Frontiers in Atherosclerosis Research ——

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Despite crucial advances in our knowledge of the pathologic mechanism, and the availability of effective diagnostic and treatment modalities, atherosclerosis remains the leading cause of mortality and morbidity. New findings have recently introduced exciting concepts that could have a major impact on the treatment of the atherosclerotic disease. Some of these concepts derive from in vivo observations using new imaging technologies, such as high-resolution magnetic resonance imaging (MRI). We will discuss the mechanisms that lead to the development of atherosclerosis and those responsible for the acute coronary syndromes (ACS).

Definition of Atherosclerosis

Atherosclerosis defines a disease of the large arteries that is characterized by the tendency to accumulate lipids, inflammatory cells, smooth muscle cells (SMCs) and extracellular matrix within the subendothelial space, and progress in an unpredictable way to different stages. The molecular and biological mechanisms involved in the initiation and progression of atherosclerotic disease have been extensively studied during the past decades, leading to the introduction of new concepts that form the base for the actual research efforts in the field of the vascular biology![1–3]

Based on the pathological and clinical characteristics of atherosclerotic plaques, the American Heart Association Committee on Vascular Lesions[4] introduced in the mid 1990s a standardized classification of distinct plaques, which was simplified by Fuster[5] (Fig 1).

Initiation of Atherosclerotic Lesions

The initiation of atherosclerotic plaque has been correlated with the presence of a number of cardiovascular risk factors (ie, hyperlipidemia, diabetes mellitus, hypertension, aging etc), leading to endothelial dysfunction. Endothelial dysfunction is characterized by increased permeability, reduced synthesis and release of nitric oxide (NO), and over-expression of adhesion molecules (such as ICAM-1, VCAM-1, selectins, etc) and chemoattractants (such as MPC-1, M-CSF, IL-1/-6, INF-α/-γ, etc). These processes facilitate the recruitment and internalization of circulating monocytes and low density lipoprotein (LDL)[2,3,6] The lipid material accumulated within the sub-endothelial space will be oxidized and trigger an inflammatory response. Furthermore, activated monocytes will release different chemotactic and proliferative growth factors that trigger SMCs to activate, migrate and proliferate. The accumulation of macrophages, SMCs, lipid and extracellular matrix results in thickening of the arterial wall that may seriously compromise the arterial lumen and blood flow (Fig 2).

Interestingly, low high density lipoprotein (HDL)-cholesterol has been recently recognized as a major cardiovascular risk factor requiring appropriate treatment (NCEP III-guidelines)[7] In fact, low plasma concentrations of HDL have been associated with elevated concentrations of soluble cellular adhesion molecules ICAM-1 and E-selectin, which were decreased by a pharmacologically induced increase in HDL-cholesterol (by fenofibrate)[8]

The classical hypothesis of atherosclerosis initiation has been challenged recently by Sata et al[9] demonstrating in a murine model that the majority of the SMCs present in the subendothelial space of atherosclerotic lesions, as well as in graft vasculopathy and restenosis following arterial injury, derive from undifferentiated bone-marrow cells and are not the expression of replication of SMCs present in the media. This important information could explain why antiproliferative agents have not completely succeeded in controlling atherosclerotic disease and could lead to the

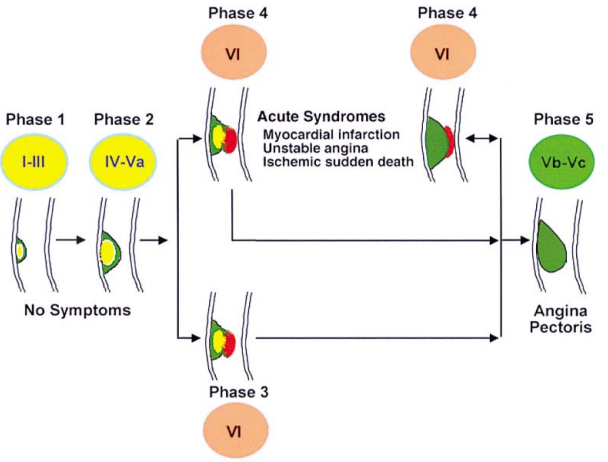

Fig 1. Schematic summary of the different stages that characterize the progression of atherosclerotic plaque (modified from Fuster et al[1]).

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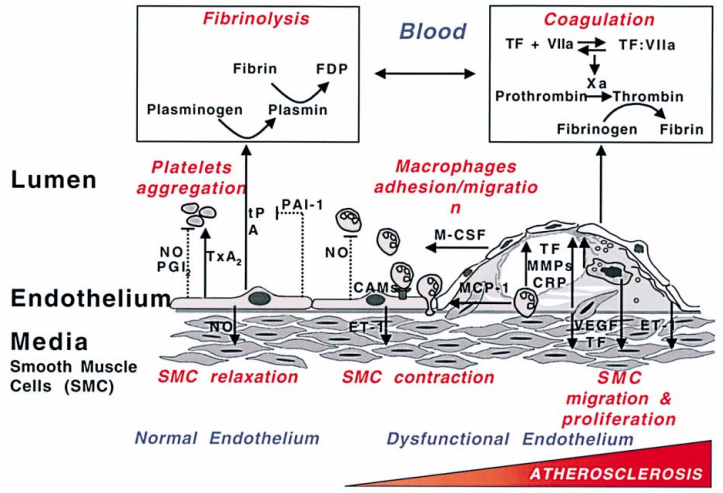

Fig 2. Progression from endothelial dysfunction to plaque disruption. Expression of endothelial adhesion molecules is induced by a variety of stimuli such as the classical cardiovascular risk factors (hyperilipidemia, diabetes, smoking etc). These molecules allow the internalization (homing) of inflammatory cells and bone marrow-derived vascular progenitor cells in the subendothelial space. Monocyte chemoattractant protein-1 is a potent chemokine, produced by the endothelial and smooth muscle cells, which causes the directed migration of leukocytes. Macrophage colony stimulating factor is an activator that can cause the expression of scavenger receptors on macrophages and a comitogen that led to the proliferation of macrophages into the forming plaque. Erosion and/or disruption of the plaque leading to thrombus formation are the major cause of acute coronary syndromes (Modified from Corti et al[36]).

development of new therapeutic approaches.

Arterial Remodeling: Revival of An Old Concept That Highlights the Role of the Media and Adventitia

The concept of arterial remodeling, introduced by Glagov et al in the late 1980s,[10] has regained importance because of recent in vivo observations using MRI. In this context, Worthley et al reported features of arterial remodeling in the natural history of atherosclerosis progression in the genetically modified rabbit lacking of LDL-receptors and constitutively presenting high levels of circulating LDL (Fig 3).[11] Our group also reported features of remodeling in ectatic coronary arteries using the same technique![12] Interestingly, crucial changes involving the media and the adventitia layers of the vessel wall, historically considered inactive structures and much less important than the endothelium in the pathogenesis of atherosclerosis, appear to be actively involved in this important step in the development of atherosclerotic disease and may even be in part responsible for the triggering mechanism leading to ACS.

Tronc et al demonstrated that increased metalloproteinases activity digesting the internal elastic lamina (IEL) modulates the process of arterial remodeling![13] More recently, Moreno et al introduced the new concept of disruption of the IEL as a trigger of plaque disruption leading to the clinical manifestation of atherosclerosis in the form of acute coronary syndromes![14] He reported a strong association between the histological evidence of IEL disruption and plaque disruption leading to thrombus formation (Fig 4). Concordantly, Burke et al demonstrated that marked expansion of the IEL occurred in plaque hemorrhages with or without rupture, whereas in erosions and total occlusions there was shrinkage of the IEL![15] Interestingly, the plaque components most strongly associated with remodeling were macrophage infiltration and the percentages of fibrous calcification and lipid core, confirming the concept of instability. The percentage of fibrous plaque area was strongly negatively associated with remodeling, whereas calcified lipid core and medial atrophy were less so.

Role of the Media and Adventitia

The role of media and adventitia has been recently highlighted by the discovery of their active involvement in the process of arterial remodeling. Media expansion following IEL disruption has been associated with outward plaque growth, plaque hemorrhage, plaque instability and even the development of arterial aneurysm. Silence et al showed, using apolipoprotein E (Apo-E) knock out mice with or without combined matrix metalloproteinase (MMP)-3 deficiency, that in mice lacking both genes atherosclerotic plaques were larger and contained more fibrillar collagen, whereas in Apo-E mice without MMP-3 deficiency, a significantly higher incidence of aortic aneurysms associated with IEL disruption was detected![16] That study confirmed the possible etiological role of atheroma in experimental aneurysm formation and highlighted the involvement of inflammatory cells as a source of proteinases, eliciting the complex pathophysiological process within the arterial wall. A further intriguing new concept in the pathogenesis of atherosclerosis derives from experimental observations of adventitial inflammation and increased plaque invasion of vasa vasorum![17–19]

Moreno et al recently associated the stage of atherosclerotic plaque with the composition of the media and adventitia, and found that disrupted plaques not only correlated with IEL disruption, but also had more medial inflammation, fibrosis and atrophy as well as more adventitial inflammation and vasa vasorum![14]

Role of the Vasa Vasorum

Increased density of vasa vasorum in the atherosclerotic coronary artery has been reported in the past in pathological studies, and an increased amount of vasa vasorum has been found in the adventitia and in the plaque itself![20–22] Proteolytic enzymes such as MMP are crucial for the invasion of the vessel wall by the vasa vasorum, a process mediated through inflammatory mechanisms.

More recently, using a novel ex vivo imaging technique (high-resolution micro-CT), the vasa vasorum structure was visualized in a volumetric (3 dimensional) fashion and an assessment was made of the effects of several interventions (such as lipid lowering and endothelin inhibition in a hypercholesterolemic pig model of atherosclerosis) on coronary vasa vasorum neovascularization![17,23,24] Hypercholesterolemia was associated with an increase in the spatial density of coronary vasa vasorum surrounding atherosclerosis

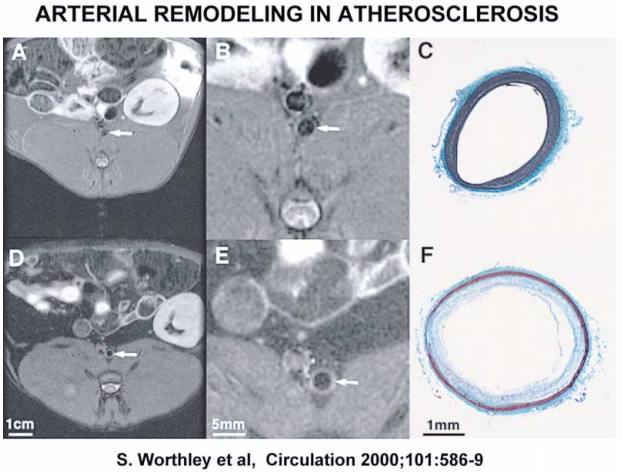

Fig 3. Arterial remodeling demonstrated in vivo using noninvasive MRI of the natural progression of atherosclerotic lesions in Watanabe heritable hyperlipidemic rabbits.[11] MR images and corresponding histopathology of atherosclerotic aortas at baseline (ie, 3 months of age) (A–C) and 6 months after balloon injury (ie, 9 months of age) (D–F). Magnified MR images of aortas in (A) and (D) clearly show the very thin aortic wall at baseline (B, arrow) and more thickened wall 6 months later (E, arrow). Furthermore, it can be appreciated that there has been no luminal loss and that the increase in vessel wall area has been outward. Corresponding histopathology sections from (A, B) and (D, E) (C and F, respectively) confirm the MR findings. It can be appreciated that there is a greater atherosclerotic burden 6 months after balloon injury; however, lumen is preserved (From Worthley et al[11]).

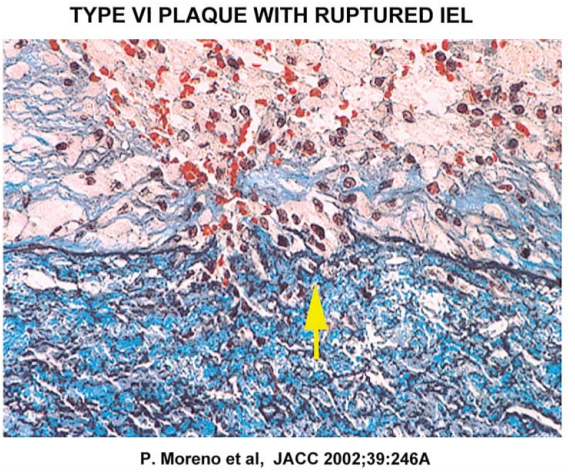

Fig 4. Increased incidence of internal elastic lamina rupture and intimal changes in complex atherosclerotic lesions. High-power histological detail of a disrupted class VI human atherosclerotic plaque showing the interface area with rupture of the internal elastic lamina (arrow). Disrupted atherosclerotic plaques, which are characterized by eccentric expansion, have larger plaque areas and higher incidence of disruption of the internal elastic lamina, suggesting an active role of the internal elastic lamina in the pathophysiology of vascular remodeling in complex atherosclerotic lesions (Courtesy of Dr P. Moreno).

rotic lesions in pigs,[17,23] and notably, this neovascularization process occurred very early in the atherosclerotic process and before epicardial endothelial dysfunction.[24] Coronary vasa vasorum neovascularization was prevented by treatment with simvastatin[25] or selective endothelin-A receptor antagonist.[26]

Progression of the Atherosclerotic Lesion and the Vulnerable Plaque

A major finding in the past 2 decades has been the recognition that plaque composition, rather than severity of stenosis, may determine the risk of thrombotic complication associated with ACS![1,27] It is now well established that atherosclerotic lesion disruption and superimposed thrombus formation play a key role in approximately 70% of the patients dying from ACS.[28,29]

Plaque disruption seems to depend on both a passive and an active phenomenon. Related to physical forces, passive plaque disruption occurs most frequently when the fibrous cap is thinnest and most heavily infiltrated by foam cells, and therefore very weak. The process of plaque disruption is, however, not purely mechanical. Inflammation has been clearly associated with all steps of the development of atherosclerotic plaques, beginning with plaque initiation and ending in plaque disruption leading to thrombus formation.[30,31] Consequently, the definition of plaque vulnerability is bound to morphological characteristics such as a large lipid content covered by a thin fibrous cap, and a high number of inflammatory cells. Activated inflammatory cells produce proteolytic enzymes (such as the metalloproteinases) able to digest the extracellular matrix and further weaken the fibrous cap. Upon disruption, the highly thrombogenic plaque content is exposed to circulating blood, which triggers thrombus formation. Changes in the geometry of the disrupted plaque, as well as organization of the mural thrombus by connective tissue, can lead to rapid plaque progression leading to more occlusive and fibrotic lesions characteristic of phase 5 type Vb or Vc lesions (Fig 1).

Thrombosis With Non-Disrupted Plaque

In one-third of ACS, particularly in acute sudden coronary death, there is no disruption of the lipid-rich high-risk (vulnerable) plaque, only a superficial erosion of a markedly stenotic and fibrotic plaque.[29] Thrombosis resulting from disruption is usually seen in plaques with a lesser degree of initial stenosis, which may not be visible, on coronary angiography. Thrombosis from endothelial erosion is usually seen at sites of pre-existing high-grade stenosis and has been reported to be more common in women and at a younger age and in men with some pro-thrombotic risk factors (smoking, diabetes, hypercholesterolemia).[32] Thus, thrombus formation in these cases in which plaque disruption is absent, may depend on a hyperthrombogenic state triggered by systemic factors, such as elevated LDL, cigarette smoking, hyperglycemia, hemostasis and others that have been associated with increased blood thrombogenicity. Diabetes mellitus, for instance is associated with platelet hyperaggregability, increased plasminogen activator inhibitor-1 (PAI-1), fibrinogen and von Willebrand's Facto, and decreased antithrombin Ⅲ activity.[33] In addition, improvement of the glycemic control is associated with a reduction in blood thrombogenicity.[34] There is significant evidence of a link between hyperlipidemia and a hypercoagulable and prothrombotic state;[35,36] this association has been substantiated by the normalization of the hypercoagulability by treatment of the hypercholesterolemic state.[36] Recently, the cardiovascular risk factors have been associated with increased activity of blood tissue factor (TF) in humans.[37]

Table 1 Potential Mechanisms of Action of High Density Lipoprotein in Atherosclerosis

Reverse cholesterol transport
Normalization of endothelial dysfunction leading to
Inhibition of monocyte adherence/infiltration
Limitation of vasoconstriction
Modulation of antithrombotic and profibrinolytic properties
Antiinflammatory effects (decreased cytokines and adhesion molecules)
Antioxidant effects (paraoxonases, PAF-AH)
Antiapoptotic effects
Antiproliferative effects

Role of Tissue Factor

Our group has shown that the thrombogenicity of disrupted atherosclerotic plaques is modulated by their TF content and, furthermore, specific inhibition of the TF pathway significantly reduces the thrombogenicity.[38] Inhibition of TF reduces thrombus formation and intimal hyperplasia after porcine coronary angioplasty.[39] Upon disruption, the atheromatous gruel, abundant in macrophages and TF, is the most thrombogenic component of the atherosclerotic plaque[40,41] and TF, the most potent trigger of the coagulation cascade, forms a high affinity complex with coagulation factors VII/VIIa leading to activation of factors IX and X, and therefore triggers both the intrinsic and extrinsic blood coagulation cascade.[42–44] Activation of the clotting cascade by TF results in the generation of thrombin, platelet activation and fibrin deposition.

The importance of TF in thrombosis has been significantly enhanced by a recent report of a blood-borne pool of TF that may play a critical role in the propagation of thrombosis.[44] Moreover, it has been reported that polymorphonuclear leukocytes might be involved in the transport of circulating TF to platelets by a CD15-dependent mechanism.[45] Higher plasma concentrations of TF antigen have been reported in ACS patients compared with those with stable angina or without coronary artery disease[46] Furthermore, circulating TF-positive microparticles with procoagulant activity have been described in patients with ACS.[47–49]

Because of the key role of TF as the initiator of the extrinsic coagulation pathway leading to clot formation after injury, specific inhibitors of its pathway have a theoretical advantage over therapies that target more 'downstream' components of the coagulation cascade.[50]

What is New About the Pathogenesis of ACS?

Apoptosis

One of the major questions is related to the source of the circulating pool of TF. Recently apoptotic phenomena have been linked to atherothrombosis and to the production of TF.[49] Early in the development of the fibrous cap, SMCs are present in significant numbers, but as the lesion progresses there is a steady decline. SMCs could vanish as a result of programmed cell death or apoptosis,[51] significantly reducing the stiffness of the fibrous cap. More recently, apoptosis of plaque macrophages has been shown to co-localize with TF expression, suggesting a potential pathogenic mechanism leading to increased plaque thrombogenicity.[52] Apoptotic death of macrophages within lesions leads to the shedding of membrane microparticles that exposes phosphatidylserine on the cell surface, conferring a potent procoagulant activity. The shed particles account for almost all the TF activity present in plaque extract and may be a major contributor in the initiation of the coagulation cascade following plaque disruption.[49]

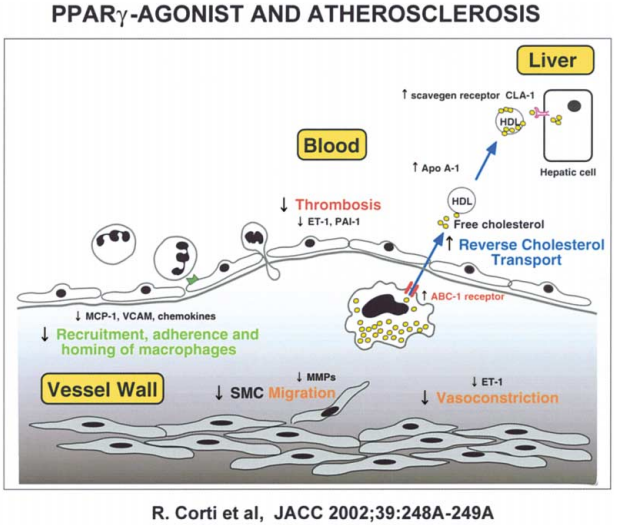

Fig 5. Potential pleiotropic effects of PPARγ-agonist that may potentially induce plaque stabilization and regression (from Corti et al[64]).

Reverse Cholesterol Transport

HDL plays an important role in the reverse cholesterol transport within the vessel wall because it is responsible for the removal of free cholesterol from the blood.[53,54] Low plasma concentration of HDL has been associated with increased cardiovascular risk, but only recently has been recognized as a major risk factor requiring adequate treatment.[7]

Several experimental studies have demonstrated the potential antiatherogenic properties of HDL: it is able to prevent plaque formation and even induce plaque regression.[55] The potential mechanisms of action of HDL have been the subject of extensive research and are summarized in Table 1. Recently, Spieker et al demonstrated that HDL administration in hypercholesterolemic patients restores normal endothelial function by increasing NO bioavailability.[56]

Peroxisomal Proliferator-Activated Receptors (PPARs)

PPARs are steroid hormone nuclear receptors that act as ligand-activated transcription factors controlling the expression of specific target genes, which in turn regulate a variety of cellular functions (Fig 5). Considering their pivotal role in atherogenesis, PPARs are considered the nuclear transcriptional regulators of atherosclerosis. Three subfamilies have been described with different tissue distribution and effects: PPAR-α, PPAR-δ and PPAR-γ. The subfamily member PPARγ plays a central role in adipogenesis and lipid metabolism, and is highly expressed in endothelial cells, SMCs, lymphocytes and macrophages.[57] PPARγ-activators may reduce plaque inflammation, inhibit expression of adhesion molecules and cytokines, and reduce production of MMPs.[58–60] Evidence indicates that PPARγ-activators can decrease thrombogenicity by reducing PAI-1 and fibrinogen concentrations, and so improve fibrinolysis.[61] In addition, PPARγ-agonist may reduce

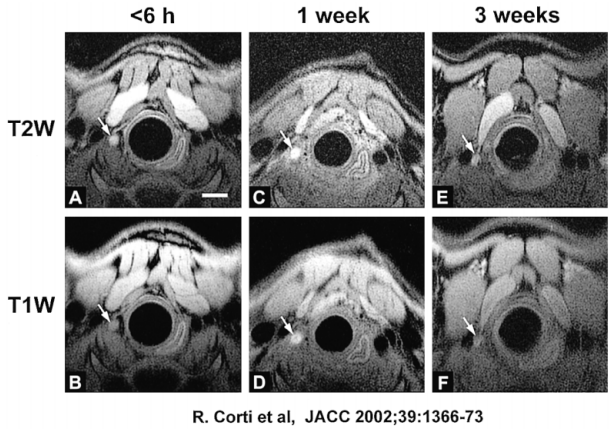

Fig 6. Arterial thrombus detection by MRI (from Corti et al[80]).

endothelin-1 production,[62] a potent vasoconstrictor and important atherogenic stimulus. Crucially, PPARγ-agonist may reduce lipid plaque content by enhancing reverse cholesterol transport, by up-regulating the genes responsible for scavenger receptor class B type I human homologue (CLA-1),[63] for adenosine triphosphate-binding cassette transporter-1 (ABC-1) and for apolipoprotein A1 (ApoA1), therefore facilitating efflux of free cholesterol from the plaque and its transport to the liver. We recently reported plaque regression and features of plaque stabilization using a new selective PPARγ-agonist in the atherosclerotic rabbit model.[64]

Role of MRI in the Detection of Atherosclerosis

High-resolution magnetic resonance (MR) has emerged as a leading in vivo imaging modality for atherosclerotic plaque characterization. It is a noninvasive technique with excellent soft-tissue contrast that differentiates plaque components on the basis of biophysical and biochemical parameters such as chemical composition and concentration, water content, physical state, molecular motion, or diffusion. MR provides imaging without ionizing radiation and, thus, can be repeated sequentially over time for serial studies. Our group has validated the use of MR for the detection and characterization of atherosclerotic plaques in vivo in different animal models and in several districts of the arterial circulation.[11,12,65–72] MR imaging permits highly accurate in vivo measurement of artery wall dimensions in human atherosclerotic carotid,[73] aortic[70] and coronary[12] lesions.

Recently we used noninvasive, high-resolution MR imaging to monitor the effects of lipid lowering by statin in asymptomatic hypercholesterolemic patients.[74] Atherosclerotic plaques were assessed with MR at different time points after initiation of lipid lowering therapy and significant regression of atherosclerotic lesions was observed. Importantly, despite the early and expected hypolipidemic effect of the statins, a minimum of 12 months was needed to observe changes in the vessel wall. There was a decrease in the vessel wall area in both carotid and aortic plaques, but no change in the lumen area at 12 months, in agreement with previous experimental studies.[11,75] The significant reduction in lesion size without affecting the lumen seems to be mediated by reduction in the plaque's lipid content,

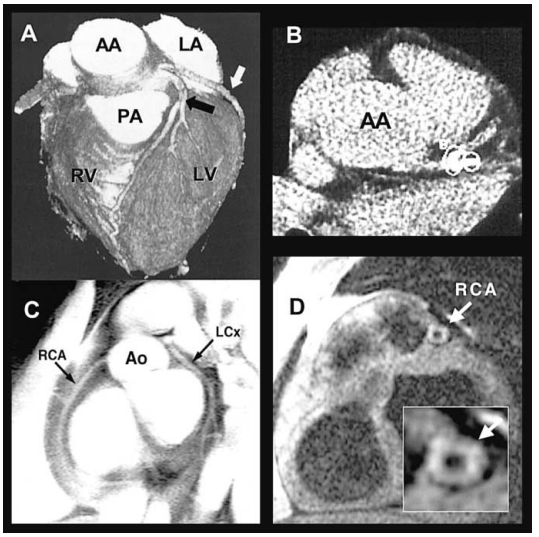

Fig 7. Nonvinvasive coronary imaging by multislice CT-angiography (A), EBCT (B), MR-angiography (C) and black blood coronary plaque imaging (D) (modified from, respectively, Achenbach et al,[98] Schmermund et al,[99] Worthley et al,[97] and Fayad and Fuster[100]).

which suggests structural changes favoring stabilization.

Considering that the propensity of plaque to disrupt is a major determinant of future ischemic events and that this propensity is increased in plaque with a high lipid content, covered by a thin fibrous cap and highly infiltrated by inflammatory cells, the ability to identify such plaques may have a major impact in the prevention of ACS by more effectively risk-stratifying the disease. MR imaging, by virtue of its ability to detect such characteristics in a noninvasive fashion, has merit because the 'Holy Grai' in coronary plaque imaging is a reliable in vivo method that can discriminate between vulnerable and stable plaque.[76]

Taking advantage of the molecular processes involved in atherothrombosis and of the rapidly evolving technology, new molecular imaging modalities have been developed and are presently actively under investigation by several research groups. It has recently been shown that it is possible to target molecules with antibodies coupled to a contrast molecule, which can be detected with MR. These molecular enhancers can potentially boost the ability of noninvasive MR imaging to detect high-risk vulnerable plaque and enhance patients' risk stratification.[77,78]

Thrombus Detection

Patients with unstable angina who suffer an event frequently suffer a second event within the following 6 weeks because the thrombus that caused the original event has not yet been organized into connective tissue and is still active, allowing propagation of the thrombus and formation of a secondary clot. Because MR is able to discriminate and characterize the plaque components on the basis of biophysical and biochemical parameters we use MRI to visualize and characterize arterial thrombi in vivo.[79] Recently, in a porcine model of obstructive carotid thrombosis we showed that MR can reliably and non-invasively detect thrombotic material in the arterial circulation and that the characteristic visual appearance of the thrombus signal intensity allows accurately definition of thrombus age (Fig 6).[80]

Therefore, MRI not only provides a method of noninva-

sively visualizing plaque and discriminating its components, but also of accurately assessing the effects of treatments, such as lipid-lowering therapy, and of timing the activity of clots and determining when they become inactive.

Coronary Imaging

Several invasive imaging techniques such as X-ray angiography, intravascular ultrasound (IVUS),[81] angioscopy,[82] infra-red light and optical coherence tomography (OCT),[83] as well as non-invasive techniques such as ultra fast computed tomography (CT) and electron beam CT,[84–87] MR and nuclear imaging techniques,[88] such as scintigraphy and positron emission tomography (PET),[89] are available to assess coronary heart disease. Most can powerfully identify the morphological features of the disease such as luminal diameter and stenosis or wall thickness, and some techniques provide an assessment of the relative risk associated with the atherosclerotic disease. However, most of these imaging techniques are incapable of characterizing the composition of the atherosclerotic plaque and therefore are unable to identify vulnerable plaques.[90]

CT and MR angiography are the most promising for the non-invasive visualization of coronary stenosis (Fig 7).[91–93] Both imaging techniques have shown promising results for the proximal portion of the vessel trees but lack of sensitivity and specificity in the mid and distal vessels presently limit their clinical applicability. In addition these angiographic techniques (as with X-ray angiography) do not provide information on the composition of the vessel wall. Therefore, high-resolution tomographic techniques allowing detailed visualization of coronary plaques have been developed,[12,67,94] although there are technical difficulties in relation to motion (both cardiac and respiratory) and the small size of the coronary arteries and their tortuous, nonlinear course.[95] In addition, high-resolution plaque imaging is a relatively time-consuming technique, limiting its applicability as screening method for the entire coronary tree. The combination of different techniques could in the future partially solve this problem. It is for instance possible that angiographic approaches may be used for mapping purposes and high-resolution images only applied selectively for the characterization of the lesion.[96]

Application to the coronary arteries suggests that MR may supply information about coronary angiography, flow velocity in addition to the characterization of the atherosclerotic lesions themselves within the coronary artery wall. Although we are still along way from this 'one-stop shop' of cardiac imaging, MR it will surely play a significant role in cardiovascular imaging in the future.[97]

Acknowledgments

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References

1. Fuster V, Fayad ZA, Badimon JJ. Acute oronary syndromes: Biology. *Lancet* 1999; **353**(Suppl 2)**:** SII5–SII9.
2. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; **104:** 365–372.
3. Lusis AJ. Atherosclerosis. *Nature* 2000; **407:** 233–241.
4. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1995; **92:** 1355–1374.
5. Fuster V, Lewis A. Conner Memorial Lecture: Mechanisms leading to myocardial infarction: Insights from studies of vascular biology. *Circulation* 1994; **90:** 2126–2146.
6. Libby P, Aikawa M. Evolution and stabilization of vulnerable atherosclerotic plaques. *Jpn Circ J* 2001; **65:** 473–479.
7. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285:** 2486–2497.
8. Calabresi L, Gomaraschi M, Villa B, Omoboni L, Dmitrieff C, Franceschini G. Elevated soluble cellular adhesion molecules in subjects with low HDL-cholesterol. *Arterioscler Thromb Vasc Biol* 2002; **22:** 656–661.
9. Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, et al. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med* 2002; **8:** 403–409.
10. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 1987; **316:** 1371–1375.
11. Worthley SG, Helft G, Fuster V, Zaman AG, Fayad ZA, Fallon JT, et al. Serial in vivo MRI documents arterial remodeling in experimental atherosclerosis. *Circulation* 2000; **101:** 586–589.
12. Fayad ZA, Fuster V, Fallon JT, Jayasundera T, Worthley SG, Helft G, et al. Noninvasive in vivo human coronary artery lumen and wall imaging using black-blood magnetic resonance imaging. *Circulation* 2000; **102:** 506–510.
13. Tronc F, Mallat Z, Lehoux S, Wassef M, Esposito B, Tedgui A. Role of matrix metalloproteinases in blood flow-induced arterial enlargement: Interaction with NO. *Arterioscler Thromb Vasc Biol* 2000; **20:** E120–E126.
14. Moreno PR, Purushothaman RK, Fuster V, O'Connor WN. Increased incidence of internal elastic lamina rupture and intimal changes in complex atherosclerotic lesions: Understanding the remodeling paradox and plaque disruption (abstract). *J Am Coll Cardiol* 2002; **39:** 247A.
15. Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R. Morphological predictors of arterial remodeling in coronary atherosclerosis. *Circulation* 2002; **105:** 297–303.
16. Silence J, Lupu F, Collen D, Lijnen HR. Persistence of atherosclerotic plaque but reduced aneurysm formation in mice with stromelysin-1 (MMP-3) gene inactivation. *Arterioscler Thromb Vasc Biol* 2001; **21:** 1440–1445.
17. Kwon HM, Sangiorgi G, Ritman EL, McKenna C, Holmes DR Jr, Schwartz RS, et al. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. *J Clin Invest* 1998; **101:** 1551–1556.
18. Ware JA. Too many vessels? Not enough? The wrong kind? The VEGF debate continues. *Nat Med* 2001; **7:** 403–404.
19. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; **6:** 389–395.
20. Barker SG, Talbert A, Cottam S, Baskerville PA, Martin JF. Arterial intimal hyperplasia after occlusion of the adventitial vasa vasorum in the pig. *Arterioscler Thromb* 1993; **13:** 70–77.
21. Booth RF, Martin JF, Honey AC, Hassall DG, Beesley JE, Moncada S. Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation. *Atherosclerosis* 1989; **76:** 257–268.
22. Martin JF, Booth RF, Moncada S. Arterial wall hypoxia following thrombosis of the vasa vasorum is an initial lesion in atherosclerosis. *Eur J Clin Invest* 1991; **21:** 355–359.
23. Jorgensen SM, Demirkaya O, Ritman EL. Three-dimensional imaging of vasculature and parenchyma in intact rodent organs with X-ray micro-CT. *Am J Physiol* 1998; **275:** H1103–H1114.
24. Herrmann J, Lerman LO, Rodriguez-Porcel M, Holmes DR Jr, Richardson DM, Ritman EL, et al. Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. *Cardiovasc Res* 2001; **51:** 762–766.
25. Wilson SH, Herrmann J, Lerman LO, Holmes DR Jr, Napoli C, Ritman EL, et al. Simvastatin preserves the structure of coronary adventitial vasa vasorum in experimental hypercholesterolemia independent of lipid lowering. *Circulation* 2002; **105:** 415–418.
26. Herrmann J, Best PJ, Ritman EL, Holmes DR, Lerman LO, Lerman A. Chronic endothelin receptor antagonism prevents coronary vasa vasorum neovascularization in experimental hypercholesterolemia. *J Am Coll Cardiol* 2002; **39:** 1555–1561.
27. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995; **92:** 657–671.

28. Zaman AG, Helft G, Worthley SG, Badimon JJ. The role of plaque rupture and thrombosis in coronary artery disease. *Atherosclerosis* 2000; **149:** 251–266.
29. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: A comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000; **20:** 1262–1275.
30. Ross R. Atherosclerosis: An inflammatory disease. *N Engl J Med* 1999; **340:** 115–126.
31. Libby P, Simon DI. Inflammation and thrombosis: The clot thickens. *Circulation* 2001; **103:** 1718–1720.
32. Farb A, Burke AP, Tang AL, Liang Y, Mannan P, Smialek J, et al. Coronary plaque erosion without rupture into a lipid core: A frequent cause of coronary thrombosis in sudden coronary death. *Circulation* 1996; **93:** 1354–1363.
33. Rauch U, Crandall J, Osende JI, Fallon JT, Chesebro JH, Fuster V, et al. Increased thrombus formation relates to ambient blood glucose and leukocyte count in diabetes mellitus type 2. *Am J Cardiol* 2000; **86:** 246–249.
34. Osende JI, Badimon JJ, Fuster V, Herson P, Rabito P, Vidhun R, et al. Blood thrombogenicity in type 2 diabetes mellitus patients is associated with glycemic control. *J Am Coll Cardiol* 2001; **38:** 1307–1312.
35. Rauch U, Osende JI, Chesebro JH, Fuster V, Vorchheimer DA, Harris K, et al. Statins and cardiovascular diseases: The multiple effects of lipid- lowering therapy by statins. *Atherosclerosis* 2000; **153:** 181–189.
36. Corti R, Badimon JJ. Value or desirability of hemorheological-hemostatic parameter changes as endpoints in blood lipid-regulating trials. *Curr Opin Lipidol* 2001; **12:** 629–637.
37. Sambola A, Hathcock J, Osende J, Nemerson Y, Fuster V, Crandall J, et al. Increased circulating tissue factor and blood thrombogenicity in type-2 diabetes (abstract). *J Am Coll Cardiol* 2002; **39:** 202A.
38. Badimon JJ, Lettino M, Toschi V, Fuster V, Berrozpe M, Chesebro JH, et al. Local inhibition of tissue factor reduces the thrombogenicity of disrupted human atherosclerotic plaques: Effects of tissue factor pathway inhibitor on plaque thrombogenicity under flow conditions. *Circulation* 1999; **99:** 1780–1787.
39. Roque M, Reis ED, Fuster V, Padurean A, Fallon JT, Taubman MB, et al. Inhibition of tissue factor reduces thrombus formation and intimal hyperplasia after porcine coronary angioplasty. *J Am Coll Cardiol* 2000; **36:** 2303–2310.
40. Fernandez-Ortiz A, Badimon JJ, Falk E, Fuster V, Meyer B, Mailhac A, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: Implications for consequences of plaque rupture. *J Am Coll Cardiol* 1994; **23:** 1562–1569.
41. Toschi V, Gallo R, Lettino M, Fallon JT, Gertz SD, Fernandez-Ortiz A, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation* 1997; **95:** 594–599.
42. Rauch U, Nemerson Y. Tissue factor, the blood, and the arterial wall. *Trends Cardiovasc Med* 2000; **10:** 139–143.
43. Nemerson Y. Tissue factor and hemostasis. *Blood* 1988; **71:** 1–8.
44. Giesen PL, Rauch U, Bohrmann B, Kling D, Roque M, Fallon JT, et al. Blood-borne tissue factor: Another view of thrombosis. *Proc Natl Acad Sci USA* 1999; **96:** 2311–2315.
45. Rauch U, Bonderman D, Bohrmann B, Badimon JJ, Himber J, Riederer MA, et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood* 2000; **96:** 170–175.
46. Soejima H, Ogawa H, Yasue H, Kaikita K, Nishiyama K, Misumi K, et al. Heightened tissue factor associated with tissue factor pathway inhibitor and prognosis in patients with unstable angina. *Circulation* 1999; **99:** 2908–2913.
47. Tedgui A, Mallat Z. Anti-inflammatory mechanisms in the vascular wall. *Circ Res* 2001; **88:** 877–887.
48. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation* 2000; **101:** 841–843.
49. Mallat Z, Tedgui A. Current perspective on the role of apoptosis in atherothrombotic disease. *Circ Res* 2001; **88:** 998–1003.
50. Corti R, Fuster V, Badimon JJ. Strategy for ensuring a better future for the vessel wall. *Eur Heart J Suppl* 2002; **4:** A31–A41.
51. Geng YJ. Biologic effect and molecular regulation of vascular apoptosis in atherosclerosis. *Curr Atheroscler Rep* 2001; **3:** 234–242.
52. Hutter R, Sauter B, Fallon JT, Fuster V, Badimon JJ. Macrophages and vascular stellate cells in human carotid plaques are prone to apoptosis and tissue factor expression (abstract). *J Am Coll Cardiol* 2001; **37:** 288A.
53. Shah PK, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: An idea whose time for testing is coming. Part II. *Circulation* 2001; **104:** 2498–2502.
54. Shah PK, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: An idea whose time for testing is coming. Part I. *Circulation* 2001; **104:** 2376–2383.
55. Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J Clin Invest* 1990; **85:** 1234–1241.
56. Spieker LE, Sudano I, Hurlimann D, Lerch PG, Lang MG, Binggeli C, et al. High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation* 2002; **105:** 1399–1402.
57. Hsueh WA, Jackson S, Law RE. Control of vascular cell proliferation and migration by PPAR-gamma: A new approach to the macrovascular complications of diabetes. *Diabetes Care* 2001; **24:** 392–397.
58. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998; **391:** 79–82.
59. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; **391:** 82–86.
60. Pasceri V, Wu HD, Willerson JT, Yeh ET. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-gamma activators. *Circulation* 2000; **101:** 235–238.
61. Marx N, Bourcier T, Sukhova GK, Libby P, Plutzky J. PPARgamma activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARgamma as a potential mediator in vascular disease. *Arterioscler Thromb Vasc Biol* 1999; **19:** 546–551.
62. Satoh H, Tsukamoto K, Hashimoto Y, Hashimoto N, Togo M, Hara M, et al. Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: A new possible role of PPAR gamma on vascular endothelial function. *Biochem Biophys Res Commun* 1999; **254:** 757–763.
63. Chinetti G, Gbaguidi FG, Griglio S, Mallat Z, Antonucci M, Poulain P, et al. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation* 2000; **101:** 2411–2417.
64. Corti R, Osende J, Fuster V, Wright SD, Fayad ZA, Diskstein E, et al. PPAR-gamma agonist induces regression of atherosclerotic plaques: In vivo study by high-resolution MRI (abstract). *J Am Coll Cardiol* 2002; **39:** 248A–249A.
65. Toussaint JF, LaMuraglia GM, Southern JF, Fuster V, Kantor HL. Magnetic resonance images lipid, fibrous, calcified, hemorrhagic, and thrombotic components of human atherosclerosis in vivo. *Circulation* 1996; **94:** 932–938.
66. Worthley SG, Helft G, Fuster V, Fayad ZA, Fallon JT, Osende JI, et al. High resolution ex vivo magnetic resonance imaging of in situ coronary and aortic atherosclerotic plaque in a porcine model. *Atherosclerosis* 2000; **150:** 321–329.
67. Worthley SG, Helft G, Fuster V, Fayad ZA, Rodriguez OJ, Zaman AG, et al. Noninvasive in vivo magnetic resonance imaging of experimental coronary artery lesions in a porcine model. *Circulation* 2000; **101:** 2956–2961.
68. Helft G, Worthley SG, Fuster V, Zaman AG, Schechter C, Osende JI, et al. Atherosclerotic aortic component quantification by noninvasive magnetic resonance imaging: An in vivo study in rabbits. *J Am Coll Cardiol* 2001; **37:** 1149–1154.
69. Fayad ZA, Fallon JT, Shinnar M, Wehrli S, Dansky HM, Poon M, et al. Noninvasive in vivo high-resolution magnetic resonance imaging of atherosclerotic lesions in genetically engineered mice. *Circulation* 1998; **98:** 1541–1547.
70. Fayad ZA, Nahar T, Fallon JT, Goldman M, Aguinaldo JG, Badimon JJ, et al. In vivo magnetic resonance evaluation of atherosclerotic plaques in the human thoracic aorta: A comparison with transesophageal echocardiography. *Circulation* 2000; **101:** 2503–2509.
71. Shinnar M, Fallon JT, Wehrli S, Levin M, Dalmacy D, Fayad ZA, et al. The diagnostic accuracy of ex vivo MRI for human atherosclerotic plaque characterization. *Arterioscler Thromb Vasc Biol* 1999; **19:** 2756–2761.
72. Helft G, Worthley SG, Fuster V, Fayad ZA, Zaman AG, Corti R, et al. Progression and regression of atherosclerotic lesions: Monitoring with serial noninvasive magnetic resonance imaging. *Circulation* 2002; **105:** 993–998.
73. Yuan C, Beach KW, Smith LH Jr, Hatsukami TS. Measurement of atherosclerotic carotid plaque size in vivo using high resolution magnetic resonance imaging. *Circulation* 1998; **98:** 2666–2671.

74. Corti R, Fayad ZA, Fuster V, Worthley SG, Helft G, Chesebro J, et al. Effects of lipid-lowering by simvastatin on human atherosclerotic lesions: A longitudinal study by high-resolution, noninvasive magnetic resonance imaging. *Circulation* 2001; **104:** 249–252.
75. Worthley SG, Helft G, Osende JI, Corti R, Fayad ZA, Fallon JT, et al. Serial evaluation of atherosclerosis with in vivo MRI: Study of atorvastatin and avasimibe in WHHL rabbits (abstract). *Circulation* 2000; **102:** II-809.
76. de Feyter PJ, Nieman K. New coronary imaging techniques: What to expect? *Heart* 2002; **87:** 195–197.
77. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, et al. In vivo magnetic resonance imaging of transgene expression. *Nat Med* 2000; **6:** 351–355.
78. Tempany CM, McNeil BJ. Advances in biomedical imaging. *JAMA* 2001; **285:** 562–567.
79. Corti R, Osende JI, Fuster V, Fayad ZA, Fallon JT, Badimon JJ. Artery dissection and arterial thrombus aging: The role of noninvasive magnetic resonance imaging. *Circulation* 2001; **103:** 2420–2421.
80. Corti R, Osende JI, Fayad ZA, Fallon JT, Fuster V, Mizsei G, et al. In vivo noninvasive detection and age definition of arterial thrombus by MRI. *J Am Coll Cardiol* 2002; **39:** 1366–1373.
81. Nissen SE, Yock P. Intravascular ultrasound: Novel pathophysiological insights and current clinical applications. *Circulation* 2001; **103:** 604–616.
82. Thieme T, Wernecke KD, Meyer R, Brandenstein E, Habedank D, Hinz A, et al. Angioscopic evaluation of atherosclerotic plaques: Validation by histomorphologic analysis and association with stable and unstable coronary syndromes. *J Am Coll Cardiol* 1996; **28:** 1–6.
83. Patwari P, Weissman NJ, Boppart SA, Jesser C, Stamper D, Fujimoto JG, et al. Assessment of coronary plaque with optical coherence tomography and high-frequency ultrasound. *Am J Cardiol* 2000; **85:** 641–644.
84. Callister TQ, Cooil B, Raya SP, Lippolis NJ, Russo DJ, Raggi P. Coronary artery disease: Improved reproducibility of calcium scoring with an electron-beam CT volumetric method. *Radiology* 1998; **208:** 807–814.
85. Detrano R, Hsiai T, Wang S, Puentes G, Fallavollita J, Shields P, et al. Prognostic value of coronary calcification and angiographic stenoses in patients undergoing coronary angiography. *J Am Coll Cardiol* 1996; **27:** 285–290.
86. Corti R, Ferrari C, Roberti M, Alerci M, Pedrazzi PL, Gallino A. Spiral computed tomography: A novel diagnostic approach for investigation of the extracranial cerebral arteries and its complementary role in duplex ultrasonography. *Circulation* 1998; **98:** 984–989.
87. Raggi P, Callister TQ, Cooil B, He ZX, Lippolis NJ, Russo DJ, et al. Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. *Circulation* 2000; **101:** 850–855.
88. Vallabhajosula S, Fuster V. Atherosclerosis: Imaging techniques and the evolving role of nuclear medicine. *J Nucl Med* 1997; **38:** 1788–1796.
89. Beanlands RS, Muzik O, Melon P, Sutor R, Sawada S, Muller D, et al. Noninvasive quantification of regional myocardial flow reserve in patients with coronary atherosclerosis using nitrogen-13 ammonia positron emission tomography: Determination of extent of altered vascular reactivity. *J Am Coll Cardiol* 1995; **26:** 1465–1475.
90. Pasterkamp G, Falk E, Woutman H, Borst C. Techniques characterizing the coronary atherosclerotic plaque: Influence on clinical decision making? *J Am Coll Cardiol* 2000; **36:** 13–21.
91. Kim WY, Danias PG, Stuber M, Flamm SD, Plein S, Nagel E, et al. Coronary magnetic resonance angiography for the detection of coronary stenoses. *N Engl J Med* 2001; **345:** 1863–1869.
92. Nieman K, Oudkerk M, Rensing BJ, van Ooijen P, Munne A, van Geuns RJ, et al. Coronary angiography with multi-slice computed tomography. *Lancet* 2001; **357:** 599–603.
93. Becker CR, Ohnesorge BM, Schoepf UJ, Reiser MF. Current development of cardiac imaging with multidetector-row CT. *Eur J Radiol* 2000; **36:** 97–103.
94. Botnar RM, Stuber M, Kissinger KV, Kim WY, Spuentrup E, Manning WJ. Noninvasive coronary vessel wall and plaque imaging with magnetic resonance imaging. *Circulation* 2000; **102:** 2582–2587.
95. van der Wall EE, Vliegen HW, de Roos A, Bruschke AV. Magnetic resonance imaging in coronary artery disease. *Circulation* 1995; **92:** 2723–2739.
96. Corti R, Fuster V, Badimon JJ, Hutter R, Fayad ZA. New understanding of atherosclerosis (clinically and experimentally) with evolving MRI technology in vivo. *Ann NY Acad Sci* 2001; **947:** 181–195; discussion 195–198.
97. Worthley SG, Farouque OHM, Helft G, Meredith IT. Coronary artery imaging in the new millennium. *Heart Lung Circ* 2002; **11:** 19–25.
98. Achenbach S, Ulzheimer S, Baum U, Kachelriess M, Ropers D, Giesler T, et al. Noninvasive coronary angiography by retrospectively ECG-gated multislice spiral CT. *Circulation* 2000; **102:** 2823–2828.
99. Schmermund A, Mohlenkamp S, Baumgart D, Kriener P, Pump H, Gronemeyer D, et al. Usefulness of topography of coronary calcium by electron-beam computed tomography in predicting the natural history of coronary atherosclerosis. *Am J Cardiol* 2000; **86:** 127–132.
100. Fayad ZA, Fuster V. Clinical imaging of the high-risk or vulnerable atherosclerotic plaque. *Circ Res* 2001; **89:** 305–316.