Cardiovascular diseases are still the primary causes of mortality in the United States and in Western Europe. Arterial thrombosis is triggered by a ruptured atherosclerotic plaque and precipitates an acute vascular event, which is responsible for the high mortality rate. These rupture-prone plaques are called “vulnerable plaques.” During the past decades, much effort has been put toward accurately detecting the presence of vulnerable plaques with different imaging techniques. In this review, we provide an overview of the currently available invasive and noninvasive imaging modalities used to detect vulnerable plaques. We will discuss the upcoming challenges in translating these techniques into clinical practice and in assigning them their exact place in the decision-making process. (J Am Coll Cardiol 2011;57:1961–79) © 2011 by the American College of Cardiology Foundation

Each year in Europe and in the United States, atherosclerotic diseases, including acute coronary syndromes (ACS) and stroke, are responsible for nearly one-half of all deaths and are among the leading causes of disability (1,2). Although atherosclerosis alone is a relatively benign disease, it is frequently complicated by acute thrombosis, usually triggered by the rupture or erosion of an atherosclerotic plaque, which in turn precipitates the acute ischemic events. Until now, identification of patients at high risk for acute vascular events has mainly relied on the estimation of their 10-year probability to present with or to die from an acute coronary or vascular problem with either the Framingham risk equation (3) or the Systematic Coronary Risk Evaluation system (4). Unfortunately, although these scores correctly estimate the cardiovascular risk of men of middle-age, they usually greatly underestimate that of younger patients (5,6). Noninvasive tests such as stress single-photon emission computed tomography (SPECT) or stress echocardiography are frequently used to refine cardiovascular risk assessment in high-risk individuals. These tests provide information on the hemodynamic significance of potential coronary artery stenoses. Accordingly, they only allow for the identification of the most severely obstructive plaques (i.e., those that limit coronary flow reserve). Because most vascular events are caused by the rupture or erosion of nonhemodynamically significant plaques, which by far outnumber flow-limiting lesions (probably by a factor of 10) (7), the predictive value of noninvasive stress testing in predicting acute vascular events is particularly poor. More recently, coronary calcium scoring (8), with computed tomography, and various inflammatory biomarkers (9) have been proposed to help risk stratify high-risk patients. Although these tests do improve our ability to identify patients at risk, their predictive value remains too low to be used in daily clinical practice.

During the past decade, development of new tools to identify “vulnerable atherosclerotic plaques” (i.e., those likely to cause an acute vascular event) has received considerable attention. It is the aim of the present review to summarize the current state of knowledge in this fast-evolving field and to discuss some of the challenges in translating this knowledge into clinical practice. This review gives a short description of the techniques, their validation, their possible clinical usefulness, and their limitations.

Pathogenesis of Vulnerable Atherosclerotic Plaques: Structural, Cellular, and Functional Features Associated With Plaque “Vulnerability”

Atherosclerotic lesions are focal thickening of the intima of arteries of large and medium size. Lipids, inflammatory and smooth muscle cells, as well as connective tissue are systematically found in these lesions. In patients with hypercholesterolemia, low-density lipoprotein (LDL) particles in excess infiltrate the artery and are retained in the intima, particularly at sites of hemodynamic strain (10). Local oxidative and enzymatic modifications of LDL particles lead to the release of inflammatory lipids that induce endothelial cells to express leukocyte adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, E-selectin, and P-selectin, which in turn facilitates the recruitment of white blood cells, including T-lymphocytes and monocytes (11,12). Monocytes recruited through the activated endothelium differentiate into macrophages. The
modified LDL particles are taken up by the scavenger receptors of these macrophages, which evolve into foam cells. Several endogenous molecules can ligate toll-like receptors on these cells, inducing their activation and leading to the release of inflammatory cytokines, chemokines, oxygen and nitrogen radicals, and other inflammatory molecules (12,13). During the progression of atherosclerosis, endothelial cells, macrophages, and smooth muscle cells die from apoptosis or necrosis (14). Disintegration of foam cells, loss of smooth muscle cells, and production of matrix metalloproteinases by activated leukocytes have detrimental consequences—leading to the formation of destabilizing lipid-rich cores and fragile and rupture-prone fibrous caps (11,15). Angiogenesis is also frequent in advanced atherosclerosis and is probably a marker of ongoing disease activity and might thus characterize high-risk plaques. There is evidence that hypoxia plays an important role in triggering microvessel proliferation in the inner layers of the vessel wall, to compensate for the limited diffusion of oxygen in large atherosclerotic plaques. The new microvessels originate from adventitial vasa vasorum. They are immature, fragile, and leaky and express cellular adhesion molecules, resulting in local extravasation of plasma proteins, erythrocytes (bleeding), and inflammatory cells (16–18).

The morphological traits typically associated with rupture-prone plaques (11,19–21) are found in lesions usually referred to as thin-cap fibroatheroma (TCFA) and are shown in Figure 1. They include a large eccentric necrotic core, occupying approximately one-quarter of the plaque area (22), a thin fibrous cap, usually <65 µm (23), heavily infiltrated with macrophages and inflammatory cells (24), spotty calcifications, and vasa vasorum proliferation. Although two-thirds of acute events result from the rupture of a TCFA, the remaining events are caused by erosion of the intimal surface, with subsequent local thrombus formation (19). Eroded lesions usually exhibit less-severe luminal narrowing, fewer calcifications, less-extensive intraplaque neoangiogenesis, and less inflammation than ruptured plaques. By contrast, they usually demonstrate more negative than positive remodeling. These characteristics make them more difficult to detect than TCFAs.

### Intravascular Assessment of Plaque Vulnerability

Intravascular imaging aims at detecting vulnerable and unstable atherosclerotic plaques in patients undergoing invasive coronary angiography. Accordingly, it can only be used in patients with a definite clinical indication for invasive angiography. Because of their invasive nature, intravascular imaging modalities are not suited to detect patients with subclinical disease (i.e., those who are still asymptomatic but are at high risk for future acute vascular events).

Table 1 provides the diagnostic criteria, histological targets, validation, clinical status, and limitations of each technique. Table 2 compares the technical characteristics of the various invasive techniques and shows the assumption that legitimizes their use in the vulnerable plaque assessment. **Intravascular ultrasound.** Intravascular ultrasound (IVUS) is one of the first techniques used to assess the morphological features of coronary plaques. Intravascular ultrasound provides real-time cross-sectional tomographic images of the examined vessel perpendicular to the long axis of the catheter. The intensity of the backscatter signal is processed into gray scale with a spatial resolution of 150 µm at a frame rate of 10 to 30 frames/s. Intravascular ultrasound provides information on the anatomical characteristics of the plaque and, to a lesser extent, on its composition (25,26). IVUS has been shown to detect features associated with plaque vulnerability, such as an eccentric pattern; the presence of an echolucent core, probably representing the lipid-rich core; positive vessel wall remodeling, defined by the expansion of the overall vessel without compromising the lumen (27); presence of thrombi (28–30); plaque length (28); lumen narrowing (29); and a spotty pattern of calcifications (31). In patients with ACS, IVUS can also evidence multiple plaque rupture sites (28,32), a feature that has been correlated with systemic inflammation as measured by C-reactive protein (33). Finally, when used serially in the same patient, IVUS can demonstrate healing of plaque rupture under medical treatment (34). Three major limitations of IVUS in identifying a vulnerable plaque must be acknowledged. First, because of its limited spatial resolution, IVUS does not permit recognition of TCFA, whose thickness is less than the spatial resolution of the systems. Second, all but one (27) of the published IVUS studies have dealt with the ultrasonic aspects of ruptured plaques, which differ from TCFA in several aspects and can hardly be used to identify vulnerable plaques (Fig. 2). Finally, gray-scale IVUS cannot accurately discriminate the elements of plaque composition, because their relationship with the original acoustic signal is distorted during the scan conversion process and can be further altered by display controls such as brightness and gain. These technical limitations will probably prevent IVUS from ever becoming a clinically useful tool in the assessment of plaque vulnerability.

**IVUS-radiofrequency analysis.** Analysis of the IVUS acoustic signal before demodulation and scan conversion...
(radiofrequency [RF] data) can overcome some of the limitations inherent to conventional gray-scale IVUS imaging (35–37). The RF data are supposed to be more directly related to the interaction of ultrasound with the tissue.

Different mathematical methods have been used for RF data analysis, including autoregressive modeling (virtual histology [VH]) (Volcano, Rancho Cordova, California), fast Fourier transformation (integrated backscatter [IB]–IVUS), and wavelet analysis (37).

In VH, the frequency spectrum is calculated with a mathematical autoregression model for each line in a region of interest and averaged over the width of the region. The results of this analysis are displayed as a color-coded map (Fig. 3) of plaque composition that is superimposed over conventional gray-scale IVUS images (38). With VH, plaque components are usually categorized into 4 tissue types: fibrous, fibrofatty, calcified necrotic, and calcified (36–38). The diagnostic accuracy of VH was validated against histology, both in ex-vivo (36) and in vivo (39) experiments. These studies have shown that the sensitivity, specificity, and predictive accuracy of VH to detect necrotic cores—the lesions more often associated with vulnerable plaques—were 67.3%, 92.9%, and 88.3%, respectively (39). Furthermore, TCFAs were found—with VH—to be more prevalent in ACS than stable angina (40). Recently, the PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) study has provided important data about the accuracy of VH to predict acute cardiac events. The presence of TCFAs assessed by VH correlated well with subsequent risk of major adverse cardiac events (hazard ratio: 3.35). Nevertheless, the authors acknowledged that, of 595 TCFAs identified by VH, only 26 were the culprit site of the subsequent event at a median follow-up of 3.4 years, underlying the low specificity of the method (41). Recent data raise questions about the accuracy of VH to detect necrotic core (42). Similar to gray-scale IVUS, VH has significant limitations that might affect its ability to indentify vulnerable plaques. For instance, VH is unable to distinguish thrombi from other plaque components, which might explain some recently conflicting data as to the size of necrotic cores in “unstable” lesions (43). Virtual histology also has limited spatial resolution, which precludes assessment of the most prominent feature of TCFAs (i.e., fibrous cap thickness). Combined use of RF data analysis and optical coherence tomography (OCT), which has a much better spatial resolution, might be useful in this regard (44,45).

Integrated backscatter IVUS is another RF technique that has been used to characterize atherosclerotic plaques. The IB values of the different tissue components can be used to generate a color-coded IB-IVUS image, such as in VH. Both ex vivo (46,47) and in vivo (48) studies have validated the accuracy of this approach. Three-dimensional reconstruction of IB values has been proposed as well, offering an attractive possibility to monitor changes in tissue characteristics over time (49).

Wavelet analysis is a mathematical model used to discriminate a unique local wave within a complex signal. Color-coded mapping of wavelet analysis allows for the detection of lipid versus fibrous plaques, both in vitro and in vivo, with a sensitivity between 81% and 83% and a specificity between 82% and 85% (50).

Intravascular elastography: palpography. Elastography measures the mechanical properties of tissue with a cross-correlation analysis of RF-ultrasound signals recorded at different pressures. For intravascular purposes, elastography
is called palpography. Palpography measures the local rate of plaque deformation (strain) in response to the pulsating force of blood pressure. A pressure differential of 4 mm Hg is large enough to strain the vessel between 0% and 1%. Palpography assesses 1 strain value/angle. The value is then plotted as a color-coded contour at the lumen vessel boundary to obtain a palpogram of the artery (51,52). When local strain is normalized by intracoronary pressure, the local modulus of elasticity can be calculated to assess vessel wall and plaque characteristics (51,52), fibrous plaques being

| Table 1 | Invasive Techniques for Imaging the Vulnerable Plaque |
|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Histological or Physiological Targets | Histological Validation (Ref. #) | Prediction of Clinical Outcomes (Ref. #) | Clinical Status | Limitations |
| IVUS | | | | |
| Eccentric pattern | | | NA | ++ + |
| Outward remodeling | | | NA | |
| Lipid core (echolucent core) | | | | |
| Plaquel area | 0.87 (26) | | | |
| Spongy calcifications | | | | |
| IVUS-RF analysis | | | | |
| Plaque composition (fibrous, fibro-fatty, calcified necrotic, and calcified tissues) | | | | |
| Identification of TCFA | | | | |
| Palpography | | | | |
| Plaque composition (fibrous, fibro-fatty) | | | | |
| Thin fibrous cap with macrophage infiltration | 0.82 (56) | | | |
| OCT | | | | |
| Plaque composition (Fibrous, fibrocalcic, lipid-rich) | | | | |
| Thin fibrous cap | | | | |
| Macrophages | 0.84 (70) | | | |
| Neoaangiogenes (OCT-Doppler) | 0.475 (72) | | | |
| Collagen content (polarization-sensitive-OCT) | | | | |
| NIRs | | | | |
| Thin fibrous cap | | | | |
| Lipid core | | | | |
| Macrophages | | | | |
| IV-MRI | | | | |
| Plaque composition (lipid, fibrous, calcified tissues) | | | | |
| Angioscopy | | | | |
| Lipid core and thin fibrous cap (yellow plaques) | | | | |
| Thermography | | | | |
| Macrophages | 0.68 (90) | | | |
| Shear-stress imaging | | | | |
| Localized “high shear stress pattern” | | | | |

See text for details.

Acc = accuracy; ACS = acute coronary syndromes; AHR = adjusted hazard ratio; AUC = area under the receiver operating curve; HR = hazard ratio; IV-MRI = intravascular magnetic resonance imaging; IVUS = intravascular ultrasound; MACE = major adverse cardiac event; NIRS = near-infrared spectroscopy; NPV = negative predictive value; OCT = optical coherence tomography; PPV = positive predictive value; RF = radiofrequency; Se = sensitivity; Sp = specificity; TCFA = thin-cap fibroatheroma; +++ = widely used in clinical routine; ++ = must be validated in larger groups of patients; + = clinical experience limited; − = currently not used for clinical assessment or research tool.
stiffer than lipid-rich plaques. The method was validated against histopathology both ex vivo (53), on explanted atherosclerotic human femoral and coronary arteries, and in vivo, in a small group of patients scheduled for percutaneous intervention (54) as well as in Yucatan pigs (55). In ex vivo experiments, good correlations were found between the presence of TCFAs or macrophage infiltration and high regional strain values (Fig. 4)(56). A strong relationship was also reported between the number of highly deformable plaques on palpography and both the clinical presentation (stable angina pectoris vs. ACS) of patients and circulating C-reactive protein levels (57,58). In vivo studies have confirmed the good correlation between strain values and histopathology (55), fatty plaques being identified with a sensitivity of 100% and specificity of 80%, respectively. Thus, these studies suggest that palpography holds considerable promise for the identification of vulnerable plaques.

Optical coherence tomography. OCT was developed for cross-sectional imaging in biological systems. OCT uses low-coherence interferometry to generate a 2-dimensional image of optical scattering with ultra-high resolution (4 to 20 μm), which constitutes a definite advantage for vulnerable plaque imaging (59–62). Because OCT uses light to create the image, it has limited tissue penetration (2 to 3 mm) and is attenuated by blood, thus requiring the use of saline flushes, occlusion balloons, or other techniques to obtain good-quality images (63). In ex vivo experiments, OCT has been shown to identify plaque morphology with a sensitivity between 71% and 96% and a specificity between

<table>
<thead>
<tr>
<th>Physical Property</th>
<th>Technique</th>
<th>Assumption</th>
<th>Energy, MHz</th>
<th>Wavelength, μm</th>
<th>Frame Rate (Frames/s)</th>
<th>Penetration</th>
<th>Thermal Resolution°</th>
<th>Spatial Resolution, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustic</td>
<td>Conventional IVUS</td>
<td>The backscattered signal is different among lipid pool, fibrous, and calcified tissues</td>
<td>Ultrasound 30–40</td>
<td>35–80</td>
<td>7–30</td>
<td>8–10 mm</td>
<td>NA</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>IVUS-RF analysis</td>
<td>RF data are supposed to be more directly related to the interaction of ultrasound with the tissue</td>
<td>Ultrasound 30–40</td>
<td>35–80</td>
<td>7–30</td>
<td>8–10</td>
<td>NA</td>
<td>100–200</td>
</tr>
<tr>
<td></td>
<td>Palpography</td>
<td>Measures the local rate of strain in response to the pulsating force of blood pressure. Fibrous and calcified plaques are stiffer than lipid-rich plaques</td>
<td>Ultrasound 20–30</td>
<td>35–80</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>200–400</td>
</tr>
<tr>
<td>Magnetic</td>
<td>IV-MRI</td>
<td>Calculates the water diffusion coefficient, which is less in lipid-rich than in fibrous plaques</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>250</td>
<td>NA</td>
<td>120</td>
</tr>
<tr>
<td>Light scattering/ absorbance</td>
<td>Angioscopy</td>
<td>Direct visualization of the endothelial surface</td>
<td>Light</td>
<td>0.4–0.8</td>
<td>NA</td>
<td>NA</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OCT</td>
<td>Uses low-coherence interferometry to generate ultra-high-resolution images</td>
<td>Near-infrared light</td>
<td>1.3</td>
<td>15–20</td>
<td>2–3 mm</td>
<td>NA</td>
<td>4–20</td>
</tr>
<tr>
<td></td>
<td>NIRS</td>
<td>Different molecules absorb and scatter near-infrared light differently allowing for the chemical characterization of biological tissues</td>
<td>Near-infrared light</td>
<td>0.8–2.5</td>
<td>NA</td>
<td>1–2 mm</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Heat production</td>
<td>Thermography</td>
<td>Plaque inflammation and neoangiogenesis produce heat measured at the surface of the plaque</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.02 °C</td>
<td>500</td>
</tr>
</tbody>
</table>

RF = radiofrequency; other abbreviations as in Table 1.
90% and 98% (64). In patients undergoing percutaneous intervention, OCT has also been shown to favorably compare with IVUS for plaque characterization (65). Interestingly, thanks to its high-resolution capabilities, OCT allows for the recognition of the features associated with TCFA (cap thickness ≤65 μm) and is more accurate than IVUS and angioscopy to identify plaque ruptures, plaque erosions, and thrombi (44,66–69). Moreover, it is important to specify that OCT is the only technique able to detect eroded plaques, thanks to its high spatial resolution. In preliminary studies, OCT was also shown to permit quantification of macrophage content in the fibrous cap (Fig. 5) (70,71). Finally, OCT-elastography, OCT-Doppler, and polarization-sensitive OCT offer further possibilities to assess plaque characteristics (72).

**Near-infrared spectroscopy.** Near-infrared spectroscopy (NIRS) is based on the absorbance of light by organic molecules. Because different molecules absorb and scatter near-infrared light differently (73), NIRS allows for the chemical characterization of biological tissues and can be used to assess lipid and protein content in atherosclerotic plaques (74). The probability of high lipid content at the interrogation site is displayed on a color scale and is termed a “chemogram” (Fig. 6). With this method, lipid-rich cores can be detected with an area under the curve of 0.86 (75). In explanted specimens of human aorta, NIRS identifies the...
histological hallmarks of plaque vulnerability, such as a lipid pool, a thin cap, and inflammatory cells with a sensitivity between 77% and 90% and a specificity between 89% and 93% (76). Preliminary data have shown that reliable information can be obtained in vivo as well (77), despite blood flow and cardiac motion. Further studies are nonetheless needed to establish the accuracy and reproducibility of the method in daily clinical practice.

**Intravascular magnetic resonance.** Pulsed field gradient magnetic resonance imaging (MRI) has recently been used to calculate the water diffusion coefficient in atherosclerotic plaques (78). Because water diffusion is less in lipid-rich than in fibrous plaques, this approach offers the opportunity to assess and quantify lipid content in atherosclerotic vessels, such as the aorta or the coronary arteries (79). The regional lipid content is color-coded and displayed over a conventional gray-scale image with a spatial resolution of 100 μm. In preliminary ex vivo studies, correlation between MRI and histology was good, with a sensitivity of 100% and a specificity of 89%. The needs to stabilize the catheter by use of an occluding balloon and to mechanically rotate the catheter are the main limitations of this new method. Yet, preliminary data obtained in vivo, in human iliac arteries, indicate that intravascular MRI might be superior to IVUS in identifying the lipid, fibrous, and calcified components of the plaques (80,81).

**Angioscopy.** Angioscopy permits direct visualization of the surface of the plaque and the detection of thrombi. In coronary angioscopic images, plaque color is evaluated and graded as white, light yellow, yellow, or intense yellow (82). Studies have shown that yellow plaques usually contain more lipids, exhibit more positive remodeling thin fibrous caps (Fig. 7), and are more often associated with intraluminal thrombi (82–84). Furthermore, the number of yellow plaques is a strong predictor of subsequent ACS (85–87). Changes in plaque color have also been used to evaluate the efficiency of therapeutic interventions, such as statins (88). The main disadvantages are the need for blood displacement (as for OCT), the subjectivity of the assessment, and the inaccessibility of small vessels. Although angioscopy has been used for several decades, it remains a research tool, and its factual accuracy/patient is poor.

**Thermography.** Thermography is based on the assumption that plaque inflammation and neoangiogenesis produce heat that can be measured at the surface of the plaque with a dedicated catheter (89). Because vulnerable plaques are more cellular and inflammatory than stable plaques, they could be detected with this technique. Ex vivo experiments on carotid samples have shown that plaque temperature correlated positively with cell density and macrophage content and inversely with plaque thickness and the distance between the cell clusters and the luminal surface (90–92).
Thermal heterogeneity has also been demonstrated in humans with ACS, the temperature difference between plaques and healthy vessel walls reaching as much as 1.5°C (93) and correlating with the morphological features associated with plaque vulnerability such as positive remodeling (94,95). The major limitation of the method relates to the blood flow “cooling effect” (89), which might require systematic interruption of blood flow to achieve consistent and reproducible results (96).

Ultrasound-derived shear stress imaging. Although localized low shear stresses have been associated with plaque formation (97), localized high shear stresses have been linked with an increased risk of plaque rupture (Fig. 8) (98). Three-dimensional IVUS assessment of local shear stress has recently been shown to be feasible and might prove to be a useful approach to identify vulnerable plaques and potential sites of upcoming rupture. Larger studies are needed.

Noninvasive Assessment of Plaque Vulnerability

Noninvasive assessment of plaque vulnerability targets a different patient population than intravascular imaging (i.e., asymptomatic individuals who have not yet presented with an acute vascular event but are at high risk to develop one). At the present stage of development, these imaging modalities are not well-suited for imaging of the coronary arteries, owing mainly to the small size and continuous movement of these imaging targets. By contrast, they can be and have already been used successfully for carotid imaging.

Table 3 provides the histological targets, validation, potential applicability for coronary arteries, and limitations of each technique.

Multidetector computed tomography. Over the past 10 years, technical improvements—including faster gantry rotation, increased number of detectors, decreased slice thickness, and use of dual x-ray sources—have considerably increased the temporal and the spatial resolution of multidetector computed tomography (MDCT) (99). Accordingly, in recent series, the diagnostic accuracy of 64-detector MDCT to identify significant coronary artery disease has reached more than 95%.

By virtue of its ability to measure local tissue attenuation, MDCT also allows imaging of the vessel wall, potentially providing insights into the characteristics and extent of intramural atherosclerosis (100,101). MDCT can also help detect some of the features associated with plaque vulnerability, such as more positive remodeling, the presence of spotty calcifications, and a lower plaque density (<30...
Hounsfield units) (102–105). In patients with ACS, the combined presence of these 3 features allows identification of the unstable lesion with a positive predictive value, a negative predictive value, and an accuracy of 95%, 60%, and 70%, respectively (104). The MDCT criteria for plaque vulnerability have been validated against VH, in ACS patients (106), and against histopathology, in patients undergoing carotid endarterectomy (107,108). Whenever identified in patients with atherosclerotic risk factors, these features have been shown to predict the occurrence of subsequent ACS (hazard ratio: 22.8) (Fig. 9) (109). Undoubtedly, MDCT is currently the first-line method in the search for vulnerable plaque.

By use of newly designed, iodine-based contrast agents that selectively accumulate into macrophages, MDCT also offers the opportunity to selectively detect plaques containing macrophages (i.e., vulnerable plaques). In a rabbit model of atherosclerosis, Hyafil et al. (110,111) demonstrated that the signal obtained with MDCT correlated well with histopathology as well as with glucose uptake as measured by positron emission tomography (PET) (Fig. 10). Although promising, these results need to be confirmed in patients with atherosclerotic risk factors. Risk-benefit studies will also be needed before MDCT can be used for the detection and longitudinal assessment of vulnerable plaques in patients, because repeated radiation exposure remains an important and worrying limitation of x-ray imaging modalities (112).

Magnetic resonance imaging. MRI evaluates the biophysical response of tissues placed in a strong static magnetic field that are transiently exposed to electromagnetic RF pulses (113). By using different contrast weightings—such as T1-weighted, T2-weighted, proton-density-weighted, and time-of-flight scanning—MRI can provide insights into the biological characteristics of the tissue of interest, such as its water, lipid, and fibrous content. Accordingly, it has been increasingly used to characterize atherosclerotic plaques. Magnetic resonance imaging is best suited for the study of large or “static” arteries, such as the carotid arteries. Because of their small dimensions and their continuous motion during data acquisition, coronary arteries remain more difficult to image. This is the main reason why most of the data on the detection of vulnerable plaques with MRI have been obtained in large arteries.

Lipid and fibrotic plaque components have been accurately quantified on T2-weighted images, in both experimental animals (114) and humans (115,116). T2-weighted images have also been used to measure the fibrous cap thickness (117)
and to identify fibrous cap ruptures (118) and intraplaque hemorrhages (119), 2 features that are commonly found in symptomatic carotid atherosclerosis (120). A good agreement between MRI and histopathology (kappa = 0.69) was found for the identification of lipid-rich necrotic cores and intraplaque hemorrhages in human carotid plaques (115). The main limitation of MRI in identifying vulnerable plaques is its relatively poor reproducibility (121,122). Alternative imaging strategies have been recently developed to overcome this limitation. These include diffusion-weighted imaging, which pictures the various water diffusion coefficients in the plaque (123), and gadofluorine-enhanced imaging, which can be used either as a blood pool agent to detect plaque neovascularization and inflammation (Fig. 11) (124,125) or as an extracellular matrix agent to enhance detection of lipid-rich plaques (126).

Magnetic resonance imaging also allows for targeted molecular imaging, which holds considerable promise for the specific characterization of the different plaque components. For instance, plaque inflammation can be targeted with ultrasmall superparamagnetic particles of iron oxide, which accumulate in plaque macrophages (127–129); plaque neoangiogenesis can be imaged with alpha(v)beta(3)-integrin-targeted paramagnetic nanoparticles or a mimetic of arginine-glycine-aspartic acid (RGD) peptide grafted to gadolinium-diethylenetriamine penta-acetic acid (130,131); VCAM-1–expressing endothelial and inflammatory cells can be detected with a dedicated agent (132); and oxidation-rich lesions can be assessed with antibodies that recognize oxidation-specific epitopes (133).

Thanks to its submillimeter resolution, MRI is thus a promising method for the simultaneous assessment of plaque morphology and composition. Nevertheless, further technical improvements are needed before it can be used to study smaller vessels such as coronary arteries. The clinical value of MRI in predicting acute vascular events also remains to be determined.

**Nuclear imaging.** Nuclear imaging modalities, such as SPECT and positron emission tomography (PET), are intrinsically designed to image systemic disorders such as atherosclerosis. Thanks to their whole-body and targeted-imaging capabilities, SPECT and PET offer the opportunity to specifically identify the various components of atherosclerotic plaques along the entire length of the arterial tree. Single-photon emission computed tomography and PET differ in several ways. Because of its better spatial resolution (4 to 5 mm vs. 1 to 1.6 cm) (134,135) and its intrinsic capability to quantify biological processes in absolute terms, PET has been used in most of the studies on nuclear imaging of atherosclerosis.

18F-labeled fluorodeoxyglucose (FDG) is currently the most validated tracer for imaging of plaque inflammation. In metabolically active cells such as activated macrophages, FDG competes with glucose for facilitated transport sites and phosphorylation by hexokinase to yield FDG-6-phosphate. Because FDG-6-phosphate has low membrane
permeability and is not a significant substrate for either the glycolytic or the glycogen synthetic pathways, it progressively accumulates in cells as the end product of the phosphorylation reaction. The intracellular accumulation of FDG-6-phosphate can then be imaged and quantified with PET. In animals with experimental atherosclerosis (136) and humans with carotid atherosclerosis (135,137), areas of high FDG uptake have been shown to co-localize with areas of macrophages accumulation, irrespective of plaque size (135) or luminal narrowing (137). The presence of high FDG uptake has also been shown to correlate with circulating markers of inflammation such as matrix metalloproteinases (138,139). In cancer patients undergoing FDG PET imaging for staging of their neoplastic disease, increased FDG uptake in large arteries was recently shown to predict subsequent acute ischemic events (140). Finally, the FDG PET signal has been shown to be highly reproducible (141,142) and to vary in amplitude with therapeutic interventions, thus suggesting a possible role in treatment monitoring (143,144). Although FDG imaging of large-artery inflammation holds considerable promise for the early identification of vulnerable plaques, imaging of the coronary arteries has been more challenging, owing mainly to the intense tracer uptake in adjacent myocardium (134,145). Adequate visualization of coronary plaque inflammation with FDG PET will probably require suppression of the myocardial FDG signal, for instance by use of low-carbohydrate, high-fat diets (Fig. 12) (140,147).

Besides glucose uptake, numerous other metabolic and signaling pathways associated with plaque vulnerability have been targeted with nuclear imaging modalities. These include the oxidation and accumulation of LDL with 125I-labeled oxidation-specific antibodies (148), matrix metalloproteinase activity with 99mTc-labeled matrix metalloprotease inhibitors (149), macrophage apoptosis with 99mTc-labeled annexin-V (150–152), and finally monocyte recruitment with either 99mTc-labeled monocyte chemotactic protein-1 (153) (a key player in the transendothelial migration of mononuclear cells)—an 18F-labeled peptide, which can be internalized by endothelial cells through VCAM-1-mediated binding (154)—or the alpha(v)beta(3)-integrin-targeted PET tracer, 18F-galacto-RGD (Fig. 13) (155).

Although nuclear imaging is currently the leading modality for the detection of vulnerable atherosclerotic plaques, repeated radiation exposure will probably limit its widespread use for the longitudinal monitoring of patients with atherosclerotic risk factors, many of whom are women of childbearing age. Thus, future studies should probably concentrate not only on demonstrating the diagnostic accuracy of these techniques but also on investigating their risk/benefit ratio.
Ultrasound imaging. Ultrasound contrast agents have paved the way for plaque characterization with ultrasound. Ultrasound contrast agents consist of acoustically active microbubbles with a diameter of 3 to 4 μm. When exposed to an ultrasound field, these microbubbles expand and contract rhythmically, producing strong backscattered signals that can be detected by conventional ultrasound systems. Furthermore, microbubbles also produce a specific nonlinear signal that helps differentiate them from surrounding tissues (25,156). Because ultrasound contrast agents are pure intravascular tracers, contrast-enhanced ultrasonography allows for the assessment of the amount of blood contained in the microvasculature within the region of interest. This principle has been exploited to semi-quantitatively assess neovascularization in carotid plaques. Significant acoustic plaque enhancement has been correlated with both histopathology (CD31 staining) (157,158) and clinical presentation (159).

<table>
<thead>
<tr>
<th>Histological or Physiological Targets</th>
<th>Histological Validation (Ref. #)</th>
<th>Prediction of Clinical Outcomes (Ref. #)</th>
<th>Already Applied for Coronary Arteries</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCT</td>
<td>Eccentric pattern, outward remodeling, spotty calcifications</td>
<td>NA</td>
<td>NA</td>
<td>HR: 22.8 (110)</td>
</tr>
<tr>
<td>Plaque composition derived from HU</td>
<td>0.73 (109)</td>
<td>Sp: 90%, Acc: 73%</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Cap thickness</td>
<td>0.88 (108)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Macroaggregates (NL177-specific contrast agent)</td>
<td>0.63 (112)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>MRI</td>
<td>Plaque composition, lipid-rich necrotic core, intraplaque hemorrhage</td>
<td>0.93 (127)</td>
<td>Se: 78%–92%, Sp: 86%–98%, Acc: 87% (116)</td>
<td>NA</td>
</tr>
<tr>
<td>Cap thickness</td>
<td>0.73 (118)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Neoangiogenesis</td>
<td>Gadofluorine-M or gadolinium</td>
<td>0.67 (125)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Neoangiogenesis</td>
<td>αvβ3-integrin-targeted paramagnetic nanoparticles</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Iron oxide nanoparticle</td>
<td>p &lt; 0.001 (128)</td>
<td>Se: 91%, Sp: 89%, Acc: 92% (128)</td>
<td>NA</td>
</tr>
<tr>
<td>Micelles containing gadolinium and antibodies binding oxidation-specific epitopes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>VCAM-1 (endothelial cells and macrophages)</td>
<td>0.93 (133)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>PET</td>
<td>Macrophage-inflammation (FDG)</td>
<td>0.81 (137)</td>
<td>NA</td>
<td>HR: 14.1 (142)</td>
</tr>
<tr>
<td>18F-labeled VCAM-1 affinity ligand</td>
<td>0.85 (138)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Neoangiogenesis-inflammation (18F-galacto-RGD targeting αvβ3-integrin)</td>
<td>0.79 (155)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>p = 0.003 (156)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>SPECT</td>
<td>Macrophages-inflammation oxidized LDL (125iodine-labeled oxidation specific antibodies)</td>
<td>0.93 (149)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Macrophage apoptosis (99mTc-labeled annexin-V)</td>
<td>0.47 (151)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Metalloproteinases activity (99mTc-MMP inhibitors)</td>
<td>0.64 (150)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Monocyte recruitment (99mTc-MCP-1)</td>
<td>0.87 (154)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Contrast-ultrasound imaging</td>
<td>Neoangiogenesis</td>
<td>0.68 (158)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>See text for details.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FDG = 18F-labeled fluorodeoxyglucose; HU = Hounsfield unit; LDL = low-density lipoprotein; MCP = monocyte chemotactic protein; MDCT = multidetector computed tomography; MMP = matrix metalloprotease; PET = positron emission tomography; SPECT = single-photon emission computed tomography; Tc = technetium; USPIOs = ultrasmall superparamagnetic particles of iron oxide; VCAM = vascular cell adhesion molecule; other abbreviations as in Table 1.
Contrast-enhanced ultrasonography also allows for molecular imaging, with specific ligands, such as monoclonal antibodies, which are attached onto the shell of the microbubble. Because microbubbles are constrained to the intravascular space, only molecular targets appearing on the endothelial surface can be imaged. When the targeted microbubbles stream through the capillary network, they are retained locally by the specific interaction between the antibody and the antigen of interest. The presence and extent of retention can then be assessed with ultrasound imaging, usually after subtraction of the signal emanating from the circulating microbubbles. This emerging application of contrast-enhanced ultrasonography has been successfully tested in animal models of atherosclerosis by...
targeting VCAM-1 and P-selectin in aortic plaques (Fig. 14) (160–162). Up to now, molecular ultrasound imaging has remained in the domain of preclinical research.

**Future Perspectives and Concluding Remarks**

The detection of plaque vulnerability in vivo is becoming a reality, thanks to the various techniques described in this review. Currently, however, there is little evidence that the presence of vulnerable plaques is associated with an increased risk of subsequent acute ischemic events (27,48,86,109,140). Therefore, before these methods can be implemented into daily clinical routine, their diagnostic and predictive accuracy need to be evaluated in large groups of patients, in multicenter, randomized, controlled trials. The PROSPECT study brought us important information, and other studies are still underway (163).

We also need to learn which of the different morphological (thin fibrous cap, macrophage content, neoangiogenesis, and the like), molecular (e.g., matrix metalloprotease-activity, caspase-activity), biological (temperature, glucose metabolism), or mechanical features of vulnerable plaques are clinically relevant to the outcome of patients. It is likely that more than
1 plaque feature will be needed to make a clinically useful assessment. In this regard, combined imaging will be necessary. Studies will also be needed to determine how the information provided by plaque imaging can be used. For instance, if the goal of imaging is to more precisely risk-stratify patients with atherosclerotic risk factors, its predictive accuracy must be better than that of the prevailing risk assessment methods, such as the Framingham Risk Score, C-reactive protein levels, or the coronary calcium score (164). Today, no data are available to indicate this is indeed the case. The target population should also be better-defined. It is indeed likely that the information gained from imaging will only surpass that of the currently available tools in low-risk or intermediate-risk populations. But in such categories, the “number needed to screen” by imaging will have to be more important. Therefore, plaque imaging must be not only accurate but also widely available and affordable. Alternatively, if the goal of imaging is to detect rupture-prone plaques before they cause an acute ischemic event, imaging must have a high positive predictive accuracy—particularly at the coronary level—so that individual rupture-prone lesions can be timely treated. For this purpose, data on the natural history of these rupture-prone lesions, including their persistence or their spontaneous healing capabilities, will also be needed (165,166). While awaiting the results of the SECRITT I (Santorini Criteria for Investigating and Treating Thin Capped Fibroatheroma) trial, which should answer some of these questions, one must recognize we are still far from the mark (167,168). If the treatment of vulnerable plaque modifies favorably the outcome of patients, it is likely that vulnerable plaque imaging will not be an elusive goal.

Finally, the question of how often testing must repeated needs to be addressed. If repeated imaging is mandatory, safety will certainly become a major issue, because most of the currently available methods use ionizing radiation, require contrast injections, or need a vascular access.
REFERENCES


45. Gonzalez N, Garcia-Garcia HM, Regar E, et al. In vivo assessment of high-risk coronary plaques at bifurcations with combined intravas-
Imaging the Vulnerable Plaque


Key Words: atherosclerosis • invasive imaging • noninvasive imaging • vulnerable plaque.