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Non-invasive detection of vulnerable coronary plaque

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Abstract

Critical coronary stenosis have been shown to contribute to only a minority of acute coronary syndromes and sudden cardiac death. Autopsy studies have identified a subgroup of high-risk patients with disrupted vulnerable plaque and modest stenosis. Consequently, a clinical need exists to develop methods to identify these plaques prospectively before disruption and clinical expression of disease. Recent advances in invasive and non-invasive imaging techniques have shown the potential to identify these high-risk plaques. Non-invasive imaging with magnetic resonance imaging, computed tomography and positron emission tomography holds the potential to differentiate between low- and high-risk plaques. There have been significant technological advances in non-invasive imaging modalities, and the aim is to achieve a diagnostic sensitivity for these technologies similar to that of the invasive modalities. Molecular imaging with the use of novel targeted nanoparticles may help in detecting high-risk plaques that will ultimately cause acute myocardial infarction. Moreover, nanoparticle-based imaging may even provide non-invasive treatments for these plaques. However, at present none of these imaging modalities are able to detect vulnerable plaque nor have they been shown to definitively predict outcome. Further trials are needed to provide more information regarding the natural history of high-risk but non-flow-limiting plaque to establish patient specific targeted therapy and to refine plaque stabilizing strategies in the future.

Key words: Atherosclerotic plaque; Magnetic resonance imaging; Multidetector row computed tomography; Single photon emission computed tomography

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INTRODUCTION

Technological advances in cardiovascular imaging in parallel with significant development in biomedical science has changed the way we assess coronary atherosclerosis. Interestingly, two very separate but intermingled concepts have emerged. In the first concept, regardless of the extent of coronary atheroma and luminal stenosis (as observed by coronary angiography), coronary pressure measurement is used to evaluate functional ischemia of the myocardium supplied by the stenotic epicardial vessel. The decision to revascularize a stenotic epicardial vessel is based on the presence or absence of a flow-limiting trans-stenotic coronary pressure gradient. The usefulness of this concept has been clinically validated[1-3]. The sec-

ond concept evolved after reports that claimed that most acute coronary events and coronary thromboses form on angiographically non-obstructive atheroma[4-6]. Standard coronary angiography often fails to identify the culprit lesion of non trans-mural acute myocardial infarction (AMI)[7]. In addition, the plaque burden, its delineation and constituents cannot be assessed by coronary angiography. These potentially lethal but mechanically non-obstructive plaques were later labeled as high-risk plaques or vulnerable plaques[8].

The extent of underlying plaque burden causing plaque rupture is a contentious issue. Early, and in fact some recent studies, have shown that coronary thrombosis and AMI are directly proportional to the severity of the coronary stenosis[9]. However, in contrast to this notion, there is also substantial evidence in the literature to believe that coronary thrombosis can develop in as many as two-thirds of cases with non-obstructive, high-risk vulnerable coronary plaques[4-6]. Regardless of the extent of underlying coronary atheroma leading to acute coronary syndromes (ACS), it is well documented that high-risk vulnerable plaques exist and are prone to rupture[10]. These plaques are generally treated conservatively and the possibility of adaptive remodeling is routinely overlooked. Most currently available diagnostic tests are unable to predict the risk of thrombosis associated with any particular lesion in the coronary arteries. Consequently, a clinical need exists to develop new techniques that are capable of identifying vulnerable plaques before disruption occurs.

VULNERABLE PLAQUE

The composition of atherosclerotic plaque is heterogeneous by nature and contains: (1) fibrocellular components [extracellular matrix and smooth muscle cells (SMCs)]; (2) lipid-cellular components (crystalline cholesterol and cholesterol esters mixed with macrophages); (3) thrombotic components (platelets and fibrin); and (4) calcium[11-14]. Vulnerable plaques that result in rupture have been now well described as thin cap fibroatheroma (TCFA). The term vulnerable plaque was first instituted by Muller *et al*[10]. These plaques contain extensive necrotic lipid core and a thin fibrous cap (< 65 μm)[14,15]. The TCFA that is prone to rupture is deficient in SMCs but contains type 1 collagen and infiltrating active macrophages. These macrophages release matrix metalloproteinases (MMPs) 1, 8 and 13 that weaken the fibrous cap and consequently result in rupture[16]. About 65%-70% of all coronary thrombi result from plaque rupture. Although the term *thin* (< 65 μm) fibrous cap is generally accepted to define vulnerable plaques, some conflicting reports have used higher thresholds (> 200 μm) to describe vulnerable plaques[17,18]. It is important to realize that that the pathological description of vulnerable plaque lacks physiological data. The histological observations are made on static and inert tissue while plaque rupture is a more dynamic process as recently reported by Abela *et al*[19]. Cholesterol expands in volume when crystallizing from a liquid to a solid, potentially leading to rupture. In addition to plaque rupture, plaque erosion and calcified nodules can also result in thrombotic plaque disruption[20]. Erosive plaques usually have intimal thickening and are composed of fibrotic tissue with a thick fibrous cap. The thick fibrous cap in contrast to a thin cap contains an abundance of SMCs, proteoglycans and type Ⅲ collagen, but very few inflammatory cells[20]. Determination of plaque characteristics is important to appreciate the pathophysiological process of atherothrombosis, and may also provide us with a means to establish risk assessment for individual plaques in individual patients. Accordingly, imaging modalities are required to reliably evaluate plaque composition and thereby allow implementation of treatment strategies to prevent adverse coronary events.

INVASIVE IMAGING MODALITIES

Invasive techniques to determine the vulnerable plaques have received more attention. In particular three different aspects of vulnerable plaques have been investigated using different invasive technologies. The first technique focuses on imaging the microanatomy of the plaque to identify the plaque components, and includes high frequency intra-vascular ultrasound (IVUS), virtual histology-IVUS®, intravascular optical coherence tomography and intravascular magnetic resonance imaging (MRI). The second set of techniques is directed at measuring metabolic activity of the plaque to predict the risk of plaque disruption, and includes intravascular thermography and elastography. The third technique relies on measuring plaque chemical composition and detailed characterization by employing near infrared reflectance or Raman spectroscopy. Although it is likely that initial prospective identification of vulnerable plaque will first be achieved by one of these competing intra-coronary technologies, several drawbacks exist in the widespread use of these technologies. Most invasive imaging modalities are novel and therefore require specific training and highly skilled staff, they are expensive to run and consequently are not feasible for routine clinical application. Readers are directed to specific reviews on invasive imaging to detect plaque[21]. This article will focus on current non-invasive modalities available to detect vulnerable plaque with especial focus on computed tomography (CT), MRI and positron emission tomography (PET) imaging.

NON-INVASIVE IMAGING TECHNIQUES

By their very nature, invasive imaging techniques are undesirable, with a lower level of patient acceptability than non-invasive alternatives, and thus face significant hurdles if they are to be accepted into routine clinical practice[22-26]. Non-invasive imaging modalities, namely CT, MRI and scintigraphic nuclear imaging techniques may provide an alternative to invasive imaging, and have shown consider-

Table 1 Characteristics of the "ideal" non-invasive imaging modality

Patient-related factors	Technical factors
Absence of ionizing radiation	Rapid image acquisition
Spacious (minimize claustrophobia)	High spatial resolution
Suitable for all (not precluded by aneurysm clips/pacemaker leads)	High contrast resolution
Absence of breath-holding	High temporal resolution
Administration of extrinsic contrast agents unnecessary	Electrocardiogram and respiratory gating
Wide range of clinical indications	Not limited by cardiac arrhythmia
	Provides both anatomic and metabolic information
	Reproducible
	Accurate

able promise in recent studies[27-32]. Continued technological advances have bridged the gap between the accuracies of these non-invasive "modern" techniques and their "traditional" invasive counterparts, with the result that the former have been accepted into routine practice for an ever-increasing spectrum of clinical conditions and indications. One of the ways to achieve increased sensitivity is through the use of nanoparticle-based molecular imaging[27,33,34]. Nanoparticle-enhanced MRI or single photon emission CT (SPECT) can identify a thrombus by detecting fibrin, confirm the presence of an inflammatory process by detecting leukocyte and macrophage infiltration, and recognize plaque angiogenesis by detecting specific integrins involved in the formation of new blood vessels[35-38]. In addition, molecules can also be used to label specific cells both *in vivo* and *ex vivo* for imaging, for example, stem cells for cardiac regeneration and lymphocytes for specific tumors. Furthermore, and in contrast to invasive imaging, molecular imaging may also be used to provide local treatments by targeting with specific therapeutic molecules. Finally, nanoparticle-enhanced MRI and PET scanners may play an important role in the development of new drugs by providing an *in vivo* assessment of the desired molecular effect of the investigational drug. This information could be very useful when deciding whether expensive phase Ⅲ trials for new pharmaceutical agents are justified.

The "ideal" non-invasive imaging modality

In order to understand the relative strengths and weaknesses of modalities in current practice, it is important to first determine the characteristics of the "ideal" non-invasive imaging modality (Table 1). Such a non-invasive modality would combine patient acceptability, acceptable clinical indications, safety, speed and high technical specification to produce unequivocal objective data upon which future management could be confidently based. It is apparent that modern techniques, while far superior to their predecessors, fall considerably short of these model standards.

MRI

Rapid innovation and development in MR technology has allowed for widespread acceptance of cardiovascular MRI as a valuable non-invasive *in vivo* imaging modality for assessment of myocardial contractility, viability and valvular function. This is due in no small part to the commercial availability of increasingly more robust coil and gradient technology in combination with novel pulse sequence design. This technique exploits differences in proton density, proton mobility, water content, chemical composition, molecular motion and diffusion to allow for exquisite soft tissue depiction, a reflection of the high contrast resolution of MRI. Most versatile at field strengths of 1.5 Tesla, this modality fulfils several of the desirable characteristics outlined in Table 1, including absence of ionizing radiation and requirement for breath-holding or extrinsic contrast agents as well as facilitating electrocardiogram (ECG) gating with high spatial and contrast resolution.

Despite the technological advances referred to above, coronary imaging has until recent times been beyond the capabilities of MRI. This reflects the diminutive size of the coronary vessels and thus poor contrast/signal to noise ratios, a short-lived "rest period" during diastole during which coronary motion is minimized, temporal restrictions imposed by the patient's ability to suspend respiration and the resultant limited spatial resolution attainable during that time period. Several of these issues have been addressed with considerable success by the introduction of T2 preparation navigator-gated and -corrected 3-dimensional segmented techniques which, although time-consuming, allow for volumetric imaging of the coronary arteries in their entirety with subsequent multiplanar reconstruction. However, coronary MR angiographic techniques remain technically demanding and suboptimal to merit widespread acceptance into the clinical arena.

HIGH RESOLUTION MRI FOR CORONARY PLAQUE IMAGING

MR angiographic evaluation of coronary arterial luminal patency has traditionally proven challenging for the reasons outlined above. One can appreciate therefore why attempts at coronary plaque imaging add an additional level of complexity, further stressing the boundaries of MRI capability. High resolution imaging using T1, T2 and proton density weighting allows for identification of

Table 2 Selected molecular imaging agents in cardiovascular disease

Biological process	Agent	Target	Imaging platform
Plaque rupture/thrombosis	Gadolinium carrying perfluorocarbon and peptides (EP-2104R)	Fibrin	MRI
	Phage display nanoparticles generated by CEST ^{99m}Tc-apcitide	Glycoprotein Ⅱb/Ⅲa receptor	SPECT
Inflammation/apoptosis	MNP + specific antibodies	E-selectin	MRI
	Monocrystalline iron oxide	ICAM/VCAM	PET
	Cross-linked iron oxide ^{18}FDG	Macrophages glucose transporter-1, hexokinase	SPECT
	^{99m}Tc-annexin	Phosphatidylserine/caspases	SPECT
	^{99m}Tc-interleukin-2	Lymphocytes	
Angiogenesis	MNP + specific antibody ^{99m}NC100692	VCAM-1/integrin $\alpha_v\beta_3$	MRI
Myocardial infarction	MNP ^{111}Indium oxide	Stem cell labeling	MRI/SPECT

MNP: Paramagnetic nanoparticle; CEST: Chemical exchange saturation transfer; MRI: Magnetic resonance imaging; SPECT: Single photon emission computed tomography; PET: Positron emission tomography; ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion molecule; ^{18}FDG: Fluorine-18 fluorodeoxyglucose.

calcium which is hypointense on all imaging sequences, lipid which is T1- and PD-hyperintense and T2-hypointense, and fibrous tissue which produces increased signal intensity on PD-weighted imaging, while it is isointense on T1 and isointense-to-hyperintense on T2-weighted imaging. These multi-contrast signal intensity "signatures" allow for characterization of various plaque components and plaque morphology on high-resolution imaging and therefore assessment of plaque vulnerability. In order to have a null signal from blood within the adjacent coronary lumen and thereby maximize contrast resolution, real-time respiratory navigated black blood fast spin echo sequences are generally utilized. Real-time slice position correction has also been employed. The accuracy of this technique has been histologically confirmed *ex vivo*[39]. This technique has also been employed to assess coronary wall thickness, with validation in both animal models and humans[40-43]. Fayad *et al*[41] reported positive remodeling and significant coronary wall thickening in patients with coronary artery disease in comparison with control patients. Such approaches may prove useful for non-invasive coronary plaque burden measurement in the absence of ionizing radiation exposure.

MOLECULAR MR FOR PLAQUE IMAGING

Evaluation of coronary plaque at the molecular level requires the addition of targeted contrast agents (Table 2). Paramagnetic gadolinium chelates are the most commonly used extracellular contrast agent for MRI. Although in nature this metal has a short half-life, this has been compensated by novel gadolinium constructs with albumin, high-density lipoprotein and liposomes[27,33,44-47]. In addition to a longer half-life, these new generation gadolinium chelates have improved affinity for adjacent protons, allowing superior imaging of the adjoining tissue. This affinity for protons is referred to as relaxivity time, R1 for longitudinal relaxivity (adjacent tissue appears bright) and R2 for transverse relaxivity (adjacent tissue appears dark).

Magnetic iron oxide is another class of paramagnetic nanoparticle (MNP) that has been used as a molecular agent for detection of plaque characteristics. Newer generations of MNP [termed monodisperse iron oxide (MION)] are pretreated with polymer coatings that offer several advantages including better *in vivo* stability and the ability to target multiple molecules by allowing stable conjugation of a variety of ligands to the nanoparticle. In addition, a cross-linked derivative of MION (CLIO) can be conjugated to near infrared fluorochromes to allow dual modality imaging with fluorescence microscopy and MRI[48,49]. These magnetofluorescent nanoparticle have been used to target specific molecules *in vivo*[49-52]. Furthermore, a longer half-life, a high relaxivity time and a small diameter allow this nanoparticle to be a useful molecular agent for plaque and myocardial imaging[53-56]. In addition to imaging static cellular markers, MNPs can be used to identify dynamic cellular targets (specific enzymes, e.g. proteases, oxidases) by using novel magnetic relaxation switches[57,58]. These magnetic relaxation switches produce a change in relaxivity time (R2) of the nanoparticle by undergoing reversible modification in structure in the presence of a specific enzyme, which is then detected by T2-weighted MRI[57,58]. These magnetic relaxation switches hold significant potential for identifying a large set of proteins relevant to clinical cardiology including troponin, brain natriuretic peptide and C-reactive protein.

After administration, the molecule-targeting nanoparticles can approach the plaque either through the lumen of the coronary artery or through the vasa vasorum in the outer vessel wall. These molecular agents can detect cell surface markers and therefore identify cells of interest. The cell surface markers are detected by attaching a targeted ligand to the nanoparticle, while cells are identified after cellular uptake and internalization of treated nanoparticles. To target and identify specific peptides or proteins, phage display screening libraries are normally used. Molecular MR approaches to image vulnerable plaque has focused on plaque thrombosis, plaque lipid content, plaque inflammation and plaque angiogenesis. To date, these molecular agents have successfully targeted several plaque components, including fibrin, cellular markers, e.g. vascular cellular adhesion molecule (VCAM),

and angiogenesis markers, e.g. integrin $\alpha_v\beta_3$.

Imaging targets of plaque rupture

Fibrin deposition on the plaque surface is the first step after endothelial disruption following plaque rupture. Therefore targeting fibrin on the plaque surface can potentially identify high-risk plaques that are prone to disruption. The first fibrin-targeting agent was perfluorocarbon nanoparticles, which contained a liquid perfluorocarbon core and an encapsulating phospholipid monolayer. These nanoparticles have the advantage of carrying > 90 000 gadolinium atoms, hence allowing superior T1-weighted contrast enhancement[59]. Fibrin can also be targeted using phage display methods, whereby fibrin-targeted gadolinium-labeled peptides are used. These peptides (e.g. EP-1873) allow MR detection of fibrin deposits on the surface of ruptured plaques with good histological correlation in an animal model of coronary thrombosis[60,61]. The new generations of fibrin specific peptides (e.g. EP-2104R) are more selective for fibrin and have demonstrated superior targeting of a thrombus *in vivo*[62]. The main limitation of fibrin-specific peptides is the small number of gadolinium atoms (only 4 atoms) that can be attached at one time, hence requiring accumulation in sufficient quantities at the site of imaging. Nonetheless, more recent chemical exchange saturation transfer (CEST) technology is now applied to nanoparticles allowing generation of stronger MR signals. CEST contrast can originate from endogenous amide or hydroxyl protons or from exchangeable sites on exogenous CEST agents. In this technology, exchangeable protons transfer magnetization to the strong signal of bulk water after irradiation. CEST contrast agents include a liposome-based nanoparticle LIPOCEST, and other paramagnetic nanoparticles PARACEST[63-66]. *In vitro* fibrin clots are targeted with the use of anti-fibrin antibody formulated with perfluorocarbon nanoparticle PARACEST contrast agents[66].

Imaging cell surface markers

One of the foremost goals of molecular imaging is to detect the early stages of vulnerable plaque formation. Inflammation plays a critical role in the initiation and pathogenesis of atherosclerosis[67]. Exposure to inflammatory cytokines leads to over-expression of cell surface adhesion molecules especially E-selectin, intercellular adhesion molecule-1 and VCAM-1. These adhesion molecules mediate adhesion and migration of leukocytes along the endothelial surface to the inflammatory site. Therefore significant attention has been paid to develop probes that can detect these activated molecules on the surface of endothelial cells.

The expression of E-selectin has been targeted *in vivo* by generation of pegylated paramagnetic liposomes formulated with anti E-selectin antibody. E-selectin could be successfully imaged in the collared carotid arteries of apolipoprotein E-deficient apoE $^{-/-}$ mice as compared with controls[37]. Several generations of MNPs have been used to target VCAM-1. More recently, a MNP phage display with linear peptide based probe was used to successfully image *in vivo* VCAM-1 expression in the aortic roots of apoE $^{-/-}$ mice[52]. Furthermore, statin treatment of these mice blunted the imaging signal by reducing accumulation of the probe in the aortic root, thereby demonstrating sufficient dynamic range to detect a treatment effect. The specificity of the MNP VCAM-1 probe has also been evaluated in *ex vivo* human carotid endarterectomy samples. Incubation of the samples with this probe resulted in co-localization of VCAM-1-expressing cells and the MNP probe on immunohistochemistry, and resulted in a reduction T2 signal[68].

Imaging cellular targets in atherosclerotic plaque

Gadofluorine is a more lipophilic chelate of gadolinium and forms micelles due to their hydrophobic fluorinated side chain[69]. This probe has been shown to accumulate in lipid rich atherosclerotic plaques in hypercholesterolemic animal models[35,70]. High density lipoprotein (HDL) plays a key role in removing excess cholesterol from the plaques, and therefore may be a suitable candidate for transfer of nanoparticles into the plaque. HDL-like nanoparticles containing gadolinium have been developed to image the plaque *in vivo*. It has been shown that these HDL-like nanoparticles accumulate in atherosclerotic plaques after their intravenous injection[44]. The rate of this contrast uptake appears to be related to the lipid and macrophage content of the plaques in apoE $^{-/-}$ mice[27]. In addition to gadolinium chelates, CLIO-MNPs have been used to image macrophages in both animal and human atherosclerotic plaques with good histological correlation[54,55,71]. Molecular targeting of macrophages could identify the presence of inflammation in the vulnerable plaques[33,44,72]. MNP-enhanced MRI has been used in a clinical trial to assess the effect of statin dose on the level of macrophage accumulation in patients with carotid atheromatosis[73].

Imaging for angiogenesis

Growing atherosclerotic plaques initiate angiogenesis to meet their increased metabolic needs. The microvessels can cause intra-plaque hemorrhage, thereby converting these plaques to high risk and prone to rupture. New vessel formation starts from the vasa vasorum in the outer wall of the arteries and is related to the key mediator of new blood vessel formation, integrin $\alpha_v\beta_3$[36]. This integrins may represent an important molecular target for diagnosing and treating angiogenesis-related diseases. In this context, an antagonist of integrin $\alpha_v\beta_3$ has been used to induce tumor regression by targeting inhibition of neovascularization both in animals and humans[74,75]. A paramagnetic liposome containing anti-$\alpha_v\beta_3$ antibodies has been used to image this integrin in an animal model[38]. Moreover, the use of an $\alpha_v\beta_3$ integrin antagonist (fumagillin) in an animal model of atherosclerosis resulted in an anti-angiogenic effect without affecting pre-existing normal blood vessels[34,74]. Other potential investigative targets for angiogenesis include vascular en-

dothelial growth factors and integrin $\alpha_1\beta_5$.

MRI has several distinct advantages over other techniques including the absence of ionizing radiation, use of significantly less nephrotoxic contrast agent, and facilitation of high spatial resolution imaging with superb soft tissue characterization. These traits can be further enhanced by the use of specific and targeted contrast agents. Molecular imaging can help detect vulnerable plaques and may provide a risk assessment of these plaques. Furthermore, novel therapeutic nanoparticles are being developed to target these high risk plaques to provide local treatment.

CARDIAC CT

In contrast to MRI, cardiac CT incurs significant ionizing radiation exposure (10-20 mSv for retrospective gated techniques and 1-10 mSv for prospective "single phase" techniques). This modality allows for rapid data acquisition at high spatial resolution, invokes less patient anxiety given its spacious, short gantry, and has fewer contraindications than MRI. However, patient cooperation with breath-holding instructions and administration of potentially nephrotoxic contrast agents are pre-requisites for diagnostic imaging.

Electron beam CT (EBCT), which featured non-mechanical movement of the electron source and fixed detectors has been replaced in recent times by multidetector row CT (MDCT) during which both the radiation source and detectors rotate during patient motion through the CT gantry. More recently, the introduction of dual-source CT methodologies have allowed for coronary imaging without the need for β blockade. Retrospective ECG gating allows coupling of MDCT data with the corresponding phase of cardiac contraction, providing multiphasic data with superior temporal resolution when compared with EBCT. This initially incurred penalties by means of increased radiation exposure although many "low dose" protocols have since been developed and accepted into routine clinical practice.

MDCT systems allow for non-invasive characterization of different plaque components[76]. Calcium can be detected with high sensitivity and has become the established means for detection and quantification of coronary artery calcification[77]. MDCT can also be used to detect non-calcified plaque in both *ex-vivo*[78] and *in vivo*[79,80] studies. The assessment of calcification within the arterial wall may provide an independent risk factor for coronary artery disease, but it fails to identify high risk vulnerable plaques. Early studies using contrast enhanced 4-slice MDCT of coronary plaques demonstrated good correlation in differentiating between soft, intermediate and calcified plaques, as compared with IVUS[81]. In another study, 4-slice MDCT demonstrated that non-calcified plaque contributed more to total plaque burden in patients with AMI in comparison to patients with stable angina[28]. More recent studies have attempted to quantify the total volume of non-calcified atherosclerotic plaque using 64-slice MDCT. One such study evaluated 50 patients some 17 mo apart and documented a mean annualized increase of 22% in plaque volume[31]. Inter-observer variability for the quantification of non-calcified plaque volumes was found to be substantial. In a separate study the same investigators reported strong correlation between CT plaque attenuation, positive remodeling and lipid content of the plaque at contrast enhanced 64-slice CT[82]. The progression of atherosclerotic lesions resulting in focal change in luminal patency is referred to as vessel wall remodeling[83]. Positive arterial remodeling is an adaptive compensatory mechanism aiming to maintain luminal patency. In contrast, negative remodeling results in luminal narrowing irrespective of the plaque volume[84-86]. It has been shown in studies that both positive remodeling and plaque lipid content determine plaque vulnerability[84,87,88]. This hypothesis was further confirmed in another clinical study where 16/64-slice CT was performed in 38 patients with ACS and 33 patients with stable angina. The investigators reported high positive predictive value for plaque vulnerability in the presence of positive remodeling, non-calcified plaque < 30 HU, and spotty calcification[89]. Low CT attenuation, positive remodeling and spotty calcification were further shown to be associated with high risk plaque in 147 patients by 64-slice CT[90]. Other investigators have reported a 97% sensitivity for 64-slice CT when compared with IVUS in detecting plaque in 26 patients who underwent both investigations, although MDCT performed less optimally in differentiating between soft and fibrous plaque composition[32]. It has been reported that CT tends to overestimate the volume of atherosclerosis compared with IVUS[91]. In a more recent study, Pflederer *et al*[92] compared morphological features of plaque in patients with ACS and stable patients using contrast-enhanced coronary dual-source CT. The culprit lesions in patients with ACS were reported to have spotty calcification, low CT attenuation, large plaque volume and higher remodeling indices, as compared to control stable lesions.

There is little doubt that future generations of MDCT scanners will allow for improved coronary arterial plaque detection and characterization. Continuing improvements in spatial and temporal resolution, combined with innovative techniques such as dual-energy CT and the non-invasive nature of CT may pave the way to making this modality highly attractive for the identification of vulnerable plaque in the wider population. High definition detectors, multi-source tube-detector configurations and flat panel detectors are likely to feature prominently in the near future.

SCINTIGRAPHIC IMAGING

Scintigraphic techniques including SPECT and PET hold the potential for superior functional and molecular atherosclerotic imaging for prediction of the risk of plaque rupture. These techniques allow study of changes at a cellular and molecular level, and have been used for clini-

cal and research purposes to study myocardial perfusion, innervation, angiogenesis, gene expression and stem cell labeling. While such metabolic information is surplus to that provided by MRI and MDCT, scintigraphic imaging techniques are limited by relatively poor spatial and temporal resolution. Both SPECT and PET involve significant ionizing radiation exposures, and as a result, these techniques also fall considerably short of the "ideal" described earlier.

Imaging cellular targets in atherosclerotic plaque

Fluorine-18 fluorodeoxyglucose (^{18}FDG) is a glucose analog that becomes concentrated in metabolically active cells. It has been suggested that its uptake within the atherosclerotic plaque is proportional to the degree of inflammation and macrophage density[93]. Preclinical studies have suggested that PET scanning can detect ^{18}FDG accumulation within the atherosclerotic plaque[29]. Rudd *et al*[30] demonstrated that human carotid plaque inflammation can be imaged with ^{18}FDG-PET and that symptomatic plaques accumulate more ^{18}FDG than asymptomatic lesions. In addition, histological examination of the excised symptomatic plaques in this study revealed heavy macrophage infiltration. This finding confirms that inflammation is present to a greater degree in symptomatic plaques. In a separate study, Tawakol *et al*[94] also showed noticeable correlation between ^{18}FDG-PET *in vivo* signals and macrophage content on histological examination after carotid endarterectomy.

Imaging proteases

Matrix degrading MMPs present in the fibrous cap of the vulnerable plaque can also provide a surrogate marker of plaque instability. Activated MMPs cause proteolysis of the extracellular matrix of the fibrous cap causing plaque remodeling and rendering it susceptible to rupture. Specific radiotracers based on inhibitors of MMPs (^{123}I-HO-I-CGS 27023A) have been generated and tested successfully in animal models[95,96]. Increased levels of macrophage or leukocyte apoptosis in the atherosclerotic plaque may contribute to plaque instability.

Imaging apoptosis

Annexin V is an endogenous protein that binds to phosphatidylserine, a negatively charged membrane phospholipid externalized to the cell surface during early cell apoptosis. ^{99m}Tc radiolabeled annexin V uptake in animal plaque has shown a good correlation with apoptosis[97]. In another similar animal model, treatment with statins resulted in a reduction in ^{99m}Tc annexin V accumulation within the plaque signifying plaque stabilization[98]. Intracellular activation of enzyme caspases is responsible for initiation of apoptosis and progress is ongoing to develop intracellular radiotracers to target these enzymes.

Imaging vasoconstricting peptides

Endothelins are 21 amino acid vasoconstricting peptides produced primarily by the endothelial cells and play a critical role in vascular homeostasis. Endothelin has three isoforms (ET-1, ET-2, ET-3), that bind to two endothelin receptors ET_A and ET_B, the latter being present on vascular endothelial cells. Elevated levels of ET-1 have been implicated in several vascular pathological processes including atherosclerosis, stent restenosis, endothelial dysfunction and angiogenesis. Positron emitting ^{18}F-labeled ET-1 has shown good receptor affinity *in vitro*[99]. ^{99m}Tc-labeled ETs have been used *in vivo* with high uptake in atherosclerotic plaques in animal models[100]. In addition, specific radiolabeled antagonists of ET receptors have been demonstrated in animal studies[101].

SPECT or PET imaging could be used to identify unstable plaque and therefore may allow target treatment of high-risk plaques regardless of their angiographic appearances. The shortcomings of PET scanning are the need for ionizing radiation, substantial background uptake by the active myocardium and poor spatial resolution; however, this has been overcome with new technological advances combining CT with a PET scanner in new hybrid CT/PET devices. The combined molecular and anatomical imaging with SPECT or PET combined with CT, MRI or echocardiography may increase anatomical localization of the radiotracer signal. At present, imaging of moving coronary arteries with SPECT and PET represents a challenge.

WHY IS PLAQUE DETECTION IMPORTANT?

Detection of vulnerable plaque may help avert subsequent acute coronary syndrome by facilitating timely preventive regional and local therapies to the coronary arteries. Identification and aggressive medical treatment of these high-risk plaques can stabilize these plaques and potentially reduce the incidence of AMI and sudden cardiac death. To date there has been no prospective clinical data available upon which to develop treatment criteria for these plaques. There are several shortcomings in current vulnerable plaque detection techniques. Most invasive and non-invasive methods of vulnerable plaque detection lack sensitivity and specificity. In addition, the natural history of vulnerable plaque is unclear and until the dynamic progression of such lesions is defined, it will be difficult to implement any coherent proven treatment strategy. Furthermore, at present there are no data to prove that interventional treatment strategies with percutaneous coronary intervention for these high risk but asymptomatic plaques are superior to conventional medical treatment. In our opinion it is therefore essential to refine the available non-invasive techniques to assess the natural progression of vulnerable plaque. An established gold-standard may then be used in a longitudinal study in patients with established coronary artery disease to truly characterize this complex and dynamic process.

CONCLUSION

Significant progress has been made in the last decade to advance our understanding of the biology of atheroscle-

rosis. This has resulted in identification of vulnerable plaques that are prone to acute thrombotic complications, which can account for the suddenness of clinical presentation in the majority of patients with coronary artery disease. A number of different novel imaging modalities have been investigated to define the specific characteristics of vulnerable plaque. However, most of these techniques are still undergoing constant refinement and cannot reliably identify vulnerable plaque in the clinical setting. It is important to realize that plaque composition is not equal to plaque vulnerability. Most of the methodologies described in this review are able to detect particular components of plaque, for example lipids and calcium. However, at present there is no definitive evidence that *in vivo* plaque composition is directly related to plaque vulnerability nor that the observed characteristics of a plaque are related to outcome. Further research is required to increase the sensitivity and specificity of these modalities to more accurately predict adverse events in the context of high-risk plaque. In our investigation of vulnerable plaque, it is essential that we do not forget to treat cardiovascular risk factors that initiate endothelial dysfunction, which remains the earliest pathological signal of atherosclerosis. In addition, peripheral blood can contain unique cells and cytokines that may also help in identifying the general population at risk for new sudden cardiovascular events. Many unanswered questions remain, but there is a real clinical imperative to understand the natural history of these non-obstructive plaques in order to better implement preventive strategies in the future.

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