Published in final edited form as:

J Nucl Cardiol. 2014 December ; 21(6): 1112–1128. doi:10.1007/s12350-014-9959-4.

Molecular Imaging of Plaque Vulnerability

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Abstract

Over the past decade significant progress has been made in the development of novel imaging strategies focusing on the biology of the vessel wall for identification of vulnerable plaques. While the majority of these studies are still in the preclinical stage, few techniques (e.g., ¹⁸F-FDG and ¹⁸F-NaF PET imaging) have already been evaluated in clinical studies with promising results. Here, we will briefly review the pathobiology of atherosclerosis and discuss molecular imaging strategies that have been developed to target these events, with an emphasis on mechanisms that are associated with atherosclerotic plaque vulnerability.

Keywords

Atherosclerosis; Molecular Imaging; Inflammation; Angiogenesis; Apoptosis

CLINICAL SCENARIO

A 60 year old man with history of hypertension presented with recurrent atypical chest pain at rest. Physical examination, laboratory values and ECG were unremarkable. He was referred for an exercise treadmill test, during which he exercised for 12 minutes on a Bruce protocol, achieving 90% of age-predicted maximum heart rate. He was asymptomatic at peak stress and the ECG was unremarkable. Two weeks later, the patient presented with a similar, albeit persistent episode of chest pain that had started 4 hours earlier. ECG was consistent with an acute inferior wall ST elevation myocardial infarction (MI). Emergent coronary angiography confirmed total occlusion of proximal right coronary artery (RCA) with evidence of coronary thrombus. In addition there was a 60% proximal left anterior descending artery (LAD) stenosis. He underwent successful percutaneous coronary

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Disclosures:

Mehran M. Sadeghi received experimental tracers from Lantheus Medical Imaging.

intervention (PCI) of the RCA with placement of a drug eluting stent. Left ventricle (LV) ejection fraction was normal with inferior hypokinesis.

SCOPE OF THE PROBLEM AND DIAGNOSTIC GAPS

Despite a remarkable decline in cardiovascular mortality over the past decade (from 343 in 2000 to 237 per 100,000 in 2009), cardiovascular disease (CVD) remains responsible for about one third of all deaths in the United States (1). Coronary heart disease is accountable for about half of CVD mortality (1), a sobering reminder of the need for more effective preventive, diagnostic and therapeutic approach to atherosclerotic diseases. Most atherosclerotic plaques are silent, producing no significant clinical symptoms throughout the life. High grade stenotic lesions may manifest as exertional angina, claudication, leg ulcer or gangrene. The most morbid complications of atherosclerosis, acute coronary syndrome (ACS), stroke and sudden death are often triggered by plaque rupture or erosion (2). Currently, the diagnostic approach to atherosclerotic diseases often consists of evaluation of luminal patency by angiography or perfusion imaging. While this approach offers valuable information about the severity and location of luminal stenosis (or its effect on distal blood flow, i.e., ischemia), it provides little information regarding features that determine plaque's propensity for rupture or erosion.

A main goal in the management of patients with stable angina is symptom relief which can be accomplished by optimization of medical therapy and revascularization in the appropriate patient. Beyond relief of symptoms, the effectiveness of coronary revascularization compared to optimal medical therapy in preventing sudden death or myocardial infarction in patients with stable coronary artery disease (CAD) remains unsettled. Evidence from the COURAGE (3) and BARI 2D (4) trials did not confirm advantage of revascularization over optimal medical therapy with regards to death or myocardial infarction except in a limited subset of patients. However, a meta-analysis of studies published over three decades suggested that coronary revascularization (often based on ischemia) in patients with non-acute coronary artery disease might reduce mortality compared to medical therapy, but has no effect on non-fatal myocardial infarction (5). The ineffectiveness of ischemia-based revascularization in preventing myocardial infarction may be explained by its focus on treating stenosis, not plaque vulnerability, highlighting an important diagnostic gap in the management of patients with CAD. A similar gap exists in the selection of patients with asymptomatic moderate carotid stenosis who might benefit from invasive treatment. The ongoing International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA trial) which seeks to define the best management strategy for patients with moderate to severe myocardial ischemia may prompt a new diagnostic and management paradigm for CAD based on plaque biology.

PATHOPHYSIOLOGY

Atherosclerosis is a dynamic chronic metabolic and inflammatory disorder of the vessel wall which begins early in life and increases in severity and frequency over time. Endothelial dysfunction and activation, subintimal accumulation and oxidative modification of LDL, and leukocyte recruitment are early events in atherogenesis. Early atherosclerotic lesions,

fatty streaks, contain lymphocytes and lipid laden macrophages. Over time, fatty streaks convert to more advanced fibroatheromatous plaques containing a necrotic core covered by a fibrous cap. The necrotic core contains apoptotic cells, cell debris and cholesterol crystals. Fibrous cap is composed of extracellular matrix (e.g. interstitial collagen) and vascular smooth muscle cells (VSMCs) (6). Plaque rupture and erosion expose the blood to tissue factor-rich subendothelial structures, precipitating focal platelet aggregation and thrombosis. This can be either asymptomatic or occlude the lumen and compromise tissue perfusion. Plaque rupture is the most common cause of acute coronary thrombosis, while erosion, observed in nearly a quarter of cases of ACS, is more common in younger subjects and females. Structurally, plaques that are at high risk of rupture are characterized by outward remodeling (which helps to preserve the lumen), a large lipid core and a thin fibrous cap (less than 65 μm thick) (2, 6-8). Other features of plaques prone to rupture include abundance of inflammatory cells, in particular in the fibrous cap, large number of apoptotic cells (monocytes/macrophages and VSMCs), and high levels of angiogenesis, formation of fragile neovessels which predispose the lesion to intra-plaque hemorrhage. The presence of “spotty” as opposed to dense calcification has also been linked to propensity to rupture. Erosion is a loss of the endothelium that typically occurs on pathological intimal thickening or fibroatheromatous lesions (8). Unlike plaques that are prone to rupture, erosion-prone plaques are rich in intimal VSMCs and proteoglycans and appear to be less inflamed, albeit there is some disagreement regarding the degree of plaque inflammation in erosion (8). A third potential nidus for thrombosis, calcified nodule protruding into the lumen, appears to be less prevalent in ACS (8).

It remains to be fully established whether and how a stable plaque is destabilized. Macrophage and VSMC apoptosis, impairment of efferocytosis (clearance of apoptotic cells by phagocytes mediated by find-me and eat-me signals originating in apoptotic cells) and actions of proteolytic enzymes and reactive oxygen species may contribute to plaque destabilization through weakening of fibrous cap and necrotic core enlargement (9). VSMC apoptosis can be induced by macrophage-derived pro-apoptotic factors (e.g., tumor necrosis factor α), cytotoxic lymphocytes, and degranulation of mast cells, in conjunction with loss of cell-cell and cell-matrix interactions and release of matrix degradation products as a result of matrix metalloproteinase (MMP) activation (9). The resulting reduction in matrix protein synthesis and excessive proteolytic degradation of matrix proteins weaken the fibrous cap. Interferon- γ , a T lymphocyte-derived cytokine, contributes to fibrous cap weakening by inhibiting collagen synthesis by the remaining VSMCs (2). In parallel, enhanced macrophage apoptosis and inhibition of macrophage autophagy in combination with reduced efferocytosis promote inflammation and necrotic core expansion through accumulation of apoptotic cells, cell debris, and lipids. The growth of neovessels originating in the adventitia helps sustain the influx of inflammatory cells in the plaque. These leaky neovessels are deficient in pericytes and can give rise to intraplaque hemorrhage. This contributes to necrotic core enlargement (through erythrocyte phospholipid deposition) and the resultant plaque destabilization. Proteolytic enzymes play a complex role in plaque stability. A number of enzymes produced by macrophages and neutrophils, including several members of the MMP family (e.g., MMP-8, -9, and -12) weaken the fibrous cap and contribute to mechanical destabilization of the plaque (9). Conversely, other members of MMP family

(e.g., MMP-2 and -3) may have a protective effect on plaque stability through regulation of VSMC migration (9).

The risk of ACS increases immediately post-MI. This may be explained by the systemic inflammatory response triggered by MI which involves the exaggerated production and release of pro-inflammatory leukocytes from the bone marrow and the spleen. Activation of β_3 adrenergic receptors expressed by mesenchymal stem cells is implicated in this process, linking systemic stress and inflammation. Endothelial activation induced by ischemic injury promotes the influx of these leukocytes into the plaque, perhaps explaining the faster growth of atherosclerotic plaques and their destabilization observed in the post-MI period (10).

MOLECULAR IMAGING

Vulnerable coronary plaques, those lesions at high risk for triggering focal thrombosis and ACS, are often localized in proximal segments of coronary arteries. Based on both in vivo and post-mortem studies it is known that multiple vulnerable plaques can be present in the coronaries of high risk subjects (11). However, not all inflamed, thin cap fibroatheromas with a large necrotic core will rupture, and many of those plaques that do rupture remain asymptomatic (the healing process can precipitate plaque progression). Focal biomechanical forces and hemodynamics, e.g., in the course of physical or mental stress, could contribute as triggers to ACS in the correct setting. Our current limited knowledge of the determinants of plaque erosion precludes accurate prospective identification of plaques that are prone to erosion. However, the characteristics of rupture-prone lesions are better defined and a number of the biological features that contribute to this process have been targeted for molecular imaging in preclinical and clinical settings. In this section we will discuss the pathophysiological aspects of atherosclerosis and molecular imaging approaches devised to detect (and quantify) these processes in vivo. The emphasis will be on mechanisms that are related to plaque vulnerability and techniques that show promise for clinical application, especially for coronary arteries (Fig. 1, Table).

Endothelial cell activation and lipid accumulation

In addition to anti-thrombotic functions, the normal endothelium is a dynamic barrier which regulates the macromolecular and leukocyte trafficking between the circulation and subendothelial layers. Derangements in this barrier, for example as a result of abnormal shear stress and systemic risk factors such as hypercholesterolemia and hypertension, facilitate the infiltration of LDL into the subendothelial space (12). Focal LDL retention and cytotoxic products generated as a result of its modification induce endothelial cell (EC) expression of adhesion molecules (e.g., vascular cell adhesion molecule-1 (VCAM-1)) which further impairs the anti-adhesive and barrier function of the endothelium (12). Molecular imaging approaches that target endothelial activation and lipid accumulation in general identify early atherogenic events, independent of plaque vulnerability. These approaches are focused on detection of either macromolecular trafficking and vessel wall retention (e.g., anti-oxidized LDL antibodies (13)) or upregulated expression of endothelial adhesion molecules (e.g., VCAM-1- targeting radiotracers and nanoparticles (14)). Although valuable as research tools in early detection of atherosclerotic lesions and potentially monitoring the effect of preventive and therapeutic measures, the application of these

approaches in clinical imaging of plaque vulnerability maybe limited and will not be discussed in detail here. It is worth pointing out that increased endothelial permeability in atherosclerosis leads to passive macromolecular retention, e.g., of gadolinium-based agents which may be used for MR imaging of atherosclerosis (15) but may also interfere with targeted imaging through non-specific uptake of imaging probes.

Inflammatory cell recruitment, differentiation, and activation

Monocyte and macrophages are abundant in atherosclerotic lesions and play a central role in various stages of atherogenesis, from plaque initiation to development of advanced lesions and plaque rupture (16). Other inflammatory cells such as dendritic cells, lymphocytes, neutrophils, and mast cells similarly are present in atherosclerotic lesions and play important roles in atherogenesis. However, their lower abundance compared to monocytes and macrophages is a challenge for their targeted imaging. Recruitment of monocytes to atherosclerotic plaques is a complex multi-step and highly regulated process which involves the interaction of multiple adhesion molecules expressed by ECs and their counterparts on monocytes as well as focal production of various chemokines, e.g., monocyte chemoattractant protein-1, which produce a directional chemoattractant gradient. Recent experimental data suggest that focal macrophage proliferation is a major contributor to macrophage accumulation in atherosclerosis (17).

At least two major subsets of functionally distinct monocytes, called classical and non-classical monocytes, have been described in humans, mouse, and other species. These subsets can be distinguished by their differential expression of cell surface markers including CD14 and CD16 in humans and Ly-6C in the mouse (18). Both monocyte subsets are shown to infiltrate into atherosclerotic lesions in the mouse, though classical monocytes are markedly more efficient (19). Molecular imaging approaches targeting the recruitment of monocytes to atherosclerotic lesions are primarily focused on direct labeling of monocytes or detection of chemokine receptor overexpression by infiltrating monocytes. For instance, ^{111}In -oxine-labeling of monocytes has been used for monitoring monocyte migration dynamics in atherosclerotic lesions in the mouse (20). Alternatively, a number of radiotracers developed based on chemokine ligands that specifically bind to individual, e.g., CCR5 (21), or multiple chemokine receptors (22), have been used for tracking chemokine receptor expression in animal models of vascular injury and atherosclerosis.

As monocyte and macrophage are the major “professional phagocytes” of the body, the detection of the phagocytic activity within the vessel wall using nanoparticles has been extensively investigated to determine the macrophage activity and extent of atherosclerotic lesions. Small (>50 nm) and ultra-small (<50 nm) superparamagnetic iron oxide particles (such as ferumoxtran-10 and ferumoxytol) as MR contrast agents (23), and an iodinated nanoparticles (N1177) as a CT contrast agent (24) have been successfully utilized to detect the enhanced phagocytic activity within monocytes/macrophage rich atherosclerotic lesions in animal models and humans. Alternatively, monocyte differentiation to macrophages in atherosclerosis is associated with phenotypic changes which are amenable to targeted imaging. These changes include upregulation of surface receptors including lectin-like oxidized LDL receptor 1 (LOX-1), scavenger receptor A, scavenger receptor BI, CD36 and

CD68 which mediate uptake of modified lipoproteins (25). Engineering of nanoparticles that are coated with antibodies or ligands specific to surface receptors expressed by activated ECs and monocytes/macrophages, such as LOX-1 (26), is another commonly utilized strategy which may lead to enhanced accumulation of the imaging agent in monocyte/macrophage rich plaques by taking advantage of both increased phagocytic activity and cell surface expression of target molecules.

Enhanced glycolytic activity of macrophages provides a unique opportunity for molecular imaging of atherosclerosis using ^{18}F -FDG PET. The widespread availability of ^{18}F -FDG as an FDA-approved radiopharmaceutical has led ^{18}F -FDG PET imaging to supersede other molecular imaging approaches in clinical studies. The reproducibility of the ^{18}F -FDG PET imaging (27) and its association with risk of plaque inflammation (28) have been shown in carotid atherosclerotic disease. Additionally, ^{18}F -FDG uptake in the vessel wall is reduced in response to therapeutic interventions, e.g., statins (29, 30). However, the normal high myocardial uptake of ^{18}F -FDG remains a major challenge to its application in coronary arterial disease. These and some of the other issues related to ^{18}F -FDG PET imaging of atherosclerosis were recently reviewed in the Journal (31) (Fig. 2).

Macrophages are highly heterogeneous and plastic cells which depending on their interactions with neighboring cells and microenvironmental cues may exert diverse pro-inflammatory, anti-inflammatory and homeostatic functions (32). Classification of macrophages as classical (or M1) and alternatively (M2) polarized macrophages is a common scheme which is based primarily on ex vivo studies in an analogy with $T_{\text{H}1}$ and $T_{\text{H}2}$ activation states of lymphocytes. The pro-inflammatory M1 polarized macrophages which produce high levels of reactive nitrogen and oxygen species and promote a $T_{\text{H}1}$ inflammatory response are critical in anti-microbial and anti-tumor defense (32). M2 polarized macrophages are associated with a $T_{\text{H}2}$ immune response and play key roles in resolution of inflammation, tissue remodeling, and also anti-parasitic defense (32). Both M1 and M2 macrophages have been identified in human and mouse atherosclerotic lesions. A recent study on human atherosclerosis noted a progressive increase in both M1 and M2 polarized macrophages throughout the progression of lesions (33). Interestingly, M1 polarized macrophages predominated over M2 polarized macrophages in the rupture-prone shoulder region of plaques, but not in fibrous cap, lipid core or adventitia (33). The predominance of M1 polarized macrophages has also been reported in patients with symptomatic carotid artery disease, suggesting a potential role of M1 polarized macrophages in plaque instability (34). There is growing interest in using ^{18}F -FDG for detection of the polarization state of macrophages in atherosclerosis. However, ex vivo experimental data have been controversial with different studies demonstrating unchanged (35) or increased (36) glucose uptake by inflammatory activation of macrophages. It has also been suggested that enhanced glucose uptake in macrophages is stimulus-dependent and not necessarily linked to the polarization state of macrophages (37). As an alternative to ^{18}F -FDG, ^{18}F -labeled mannose (2-deoxy-2- ^{18}F -fluoro-D-mannose) has been introduced recently for targeting of plaque inflammation (38). The higher expression of mannose receptors on M2 macrophages raised the possibility that this agent may help identify this subset of macrophages, though the relative contribution of mannose receptor, versus hexose transporters, to mannose uptake needs to be determined in macrophages.

Matrix remodeling and protease activation

Excessive extracellular matrix degradation as a result of enhanced production and activation of proteases can trigger atherosclerotic plaque vulnerability by promoting VSMC apoptosis and fibrous cap thinning. MMPs and cathepsins are two major classes of proteases that play crucial roles in plaque progression and vulnerability and have been targeted for molecular imaging of plaque vulnerability. Of the cysteine protease family of cathepsins, several members, including cathepsins B, S and K, are expressed by macrophages, VSMCs and ECs in atherosclerotic lesions. These proteases have divergent effects in atherogenesis and some may contribute to plaque vulnerability through their collagenolytic and elastolytic activities (39). The cathepsin activity in atherosclerosis can be detected using cathepsin-specific activatable fluorescent probes in conjunction near infra-red fluorescent imaging. A number of studies have localized the cathepsin signal to macrophage-rich areas of the plaque, suggesting a potential role for cathepsin imaging in detecting plaque inflammation (40, 41). While the limited depth of penetration of fluorescence imaging limits the application of activatable cathepsin-specific probes in non-invasive imaging of human atherosclerosis, this approach may have a role in detecting vulnerable plaque by intravascular imaging in the course of invasive angiography.

MMPs are produced as inactive pro-enzymes by monocytes/macrophages, activated ECs and VSMCs. These secreted or membrane-bound zinc-dependent endopeptidases become active upon proteolytic or allosteric activation. MMP activity in the vessel wall is a function of MMP expression level, activation state and presence of inhibitors, including tissue inhibitors of MMPs (TIMPs). As for cathepsins, an activatable fluorescent probe which generates fluorescent light upon cleavage of a specific substrate has been used for optical imaging of MMP activity in atherosclerosis (42). Radiolabeled small molecules with high affinity for activated MMPs provide the opportunity to noninvasively detect and quantify MMP activation by SPECT in vivo (43). The feasibility of this approach is demonstrated in preclinical studies which have linked MMP signal to vessel wall inflammation and macrophage burden in animal models of atherosclerosis (44-48). Importantly, MMP imaging can track vessel wall inflammation in response to therapeutic interventions, including dietary modification or lipid lowering medications, suggesting the feasibility of a similar application in humans (46) (Fig. 3). Alternatively, P947, a gadolinium-labeled agent with moderate affinity for MMPs as well as several other proteases, including angiotensin-converting enzyme, endothelin-converting enzyme, neutral endopeptidase and aminopeptidases has been used for noninvasive MR imaging of atherosclerosis in animal models (49, 50). As for P947, most existing MMP tracers have broad MMP specificity. New radiotracers with specificity to individual members or classes of MMPs are under development and given the divergent roles of various MMPs in plaque development and progression may provide further information on vessel wall biology in atherosclerosis.

Cell death and apoptosis

Apoptosis contributes to plaque destabilization and autopsy data have shown significantly higher number of apoptotic cells in ruptured human plaques (51). Externalization of phosphatidylserine, a plasma membrane aminophospholipid normally expressed on the inner side of the plasma membrane, is one of the earliest events in apoptosis. Annexin A5 binds

with high affinity to phosphatidylserine and has been proposed as a probe for in vivo imaging of apoptosis in animal models of atherosclerosis and in humans (52). There are several pitfalls to annexin A5-based imaging of apoptosis, including phosphatidylserine externalization and thereby annexin A5 binding in non-apoptotic activated mast cells, lymphocytes and platelets, binding to viable non-apoptotic cells (53, 54), and slow clearance (55) which limit its potential utility in imaging plaque vulnerability. In addition, annexin A5 cannot differentiate between apoptotic and necrotic cell death. More specific approaches, particularly detection of caspase activity, have been investigated to overcome the limitation of annexin A5 imaging (55). Caspase cysteine-aspartate proteases are considered as executioners of apoptosis and their highly regulated activation is crucial in apoptosis. Detection of caspase-3 activation is particularly interesting as it represents the convergence point of both intrinsic and extrinsic apoptotic pathways (56). Cell membrane permeable peptides and small molecules have been developed for in vivo optical (55) and nuclear (57) imaging of caspase-3 activity and used for early identification of response to chemotherapy in tumors (57, 58). However, these caspase-targeted agents have yet to be studied in atherosclerosis.

Neovascularization

Intra-plaque neovascularization is another histological hallmark of vulnerable plaques. Autopsy data suggest increased microvessel density in ruptured plaques, thin capped plaques with intense macrophage infiltration, and lesions with intra-plaque hemorrhage compared to stable plaques (59, 60). Intra-plaque hemorrhage triggers monocyte/macrophage infiltration and plaque expansion through deposition of erythrocytes, free cholesterol and phospholipids. This sudden expansion and changes in the plaque composition are thought to be critical factors in plaque destabilization (8, 60).

The integrin $\alpha v \beta 3$ is highly expressed on proliferating and activated ECs and is often used as a marker of angiogenesis. The tripeptide arginine-glycine-aspartate (RGD) constitutes the integrin recognition site of a large number of extracellular matrix and cell surface proteins and mediates their cellular attachment. Labeled RGD peptides and peptidomimetics have been extensively investigated for molecular imaging of angiogenesis and targeted therapy in atherosclerosis (61, 62). Integrin $\alpha v \beta 3$ expression on macrophages (63) reduces the specificity of this approach for imaging angiogenesis, but may magnify its potential for imaging plaque vulnerability. Whether this approach which is now in early stages of clinical evaluation will prove to be effective in imaging plaque vulnerability remains to be determined (64) (Fig. 4).

Intra-plaque release of vascular endothelial growth factor (VEGF) and overexpression of its receptors by angiogenic vessels provide other mechanistic targets for detection of angiogenesis in atherosclerosis (65). The lack of specificity of VEGF and VEGF receptors for neovascularization raises a similar limitation (and possibly potential) as for $\alpha v \beta 3$ -targeted imaging of plaque vulnerability (66). With identification of other important angiogenic signaling molecules and pathways, e.g., CD40 and its ligand CD40L, novel imaging strategies for detection of plaque neovascularization are under development (67).

Plaque calcification

Calcification is a key feature of atherosclerotic lesions and quantification of coronary calcium deposits by CT, i.e., calcium score, is a well-established clinical marker of the overall atherosclerosis burden and predictor of future coronary events. Accumulation of macrophages in the lipid core of plaque and their release of matrix vesicles when they undergo apoptosis promote calcification (68, 69). Macrocalcification detectable by CT is generally not associated with plaque vulnerability and is rather indicative of global atherosclerotic burden. On the other hand, microcalcification and active foci of calcification may mechanically destabilize the plaque at the interface between high and low density tissues and predispose a plaque to micro-fracture and rupture (69). ^{18}F -sodium fluoride (NaF), an FDA approved radiotracer which deposits in foci of bone remodeling (70), has been used for imaging atherosclerotic plaques in human aorta, carotid, and more recently coronary arteries with promising results (71-73). Coronary uptake of ^{18}F -NaF is associated with a high Framingham risk score and history of prior cardiovascular events (72). Recently, it was reported that in patients with recent ACS ^{18}F -NaF PET may identify the culprit lesion (73) (Fig. 5). A major advantage of ^{18}F -NaF imaging compared to ^{18}F -FDG is its low myocardial uptake. However, the biological determinants and correlates of ^{18}F -NaF uptake in atherosclerosis and its role in prospective identification of high risk plaques remain to be determined.

ALTERNATIVE OR COMPLEMENTARY DIAGNOSTICS

Luminal patency (or stenosis) remains the focus of conventional vascular imaging. However, with recent advances in imaging technology and greater recognition of the limitations of angiography, there is growing interest in assessment of atherosclerotic plaque structure and composition using non-invasive (CT, MRI) or invasive high resolution imaging modalities. In this regard, several aspects of plaque structure which are readily detectable using existing CT technology (low attenuation, expansive remodeling and possibly microcalcification) have shown promise as high-risk markers for ACS (74, 75). Emerging CT technologies, including dual energy and spectral CT, may further advance the potential of CT for plaque characterization. MRI-based plaque characterization (e.g., to detect fibrous cap integrity and intra-plaque hemorrhage) is an alternative promising approach for carotid (but not yet coronary) artery disease (76). Intravascular ultrasound and “virtual histology”, optical coherence tomography, near-infrared spectroscopy and thermography are examples of existing and emerging invasive technologies which can be used to detect plaque morphology and composition and to identify determinants of vulnerability (77). While an exhaustive discussion on these invasive modalities is beyond the scope of this review, the data from PROSPECT trial are noteworthy. This prospective study in patients with ACS who underwent three-vessel coronary angiography and gray-scale and radiofrequency intravascular ultrasonographic imaging after PCI identified plaque burden of 70% or greater, minimal luminal area of 4.0 mm^2 or less, and thin-cap fibroatheroma as characteristics of non-culprit plaques that predispose to high rate of recurrent events within a relatively short follow up period (78). While such plaques were at high risk for sustaining future events, they constituted only a small fraction of thin cap fibroatheromas. A possible conclusion from these data could be that better identification of

high risk, vulnerable plaques through molecular imaging could justify invasive therapy targeted at these lesions. Ultimately, although these tools are powerful and promising, their routine clinical applicability is likely to be limited given their invasive nature and their focus mostly on anatomic structure rather than biology. Nevertheless, selected patients undergoing angiography might benefit from assessment of plaque vulnerability during the index procedure. The recent PRAMI data which support “preventive” PCI of non-culprit lesions at the time of primary PCI for ACS are intriguing (79). Whether this study will lead to more wide-spread invasive detection of plaque vulnerability in the future to narrow down the candidate lesions for PCI is yet unknown.

PITFALLS, BARIERS AND FUTURE DIRECTIONS

While a few emerging molecular imaging approaches have been successfully tested in preliminary clinical studies, the majority of such imaging studies have been performed in animal models. Inter-species differences in pathophysiology of atherosclerosis and the lack of well-accepted animal models of vulnerable plaque should be carefully considered while extrapolating animal data to humans. Biological activity of targeted tracers can be a concern, especially for non-nuclear techniques that require higher payloads of labeled molecules for imaging. For coronary imaging, the small size of coronary arteries, cardiac motion, and potential myocardial uptake of tracers are examples of other major barriers to clinical translation. New tracers need to be safe and effective, and secure approval of regulatory agencies. The substantial costs of developing and validating new tracers warrant investment in those tracers with broad potential clinical application, possibly beyond vascular imaging. Given the complexity of vascular biology, targeted imaging of individual aspects of atherosclerosis in isolation may fail to fully reflect clinical risk. Multimodality imaging with simultaneous detection of complementary biological processes as well as anatomical imaging (e.g., using CT or MRI) may provide a more comprehensive picture of the vessel wall biology and plaque vulnerability. However, the potential advantages of comprehensive information are counter-balanced with economic realities and other practical issues of clinical imaging, including radiation exposure. As our understanding of plaque vulnerability and its diagnosis evolves, we would have to grapple with the pragmatic applicability of this technology for our patients, especially given the current economic climate in the health industry and reimbursement structure. Whether this tool would allow for selecting patients who might benefit from pre-emptive PCI or emerging targeted therapies needs to be seen. Non-invasive imaging, however, would likely be a cornerstone to assess the efficacy of aggressive medical therapy in longitudinal follow-up care of these patients. Convincing large scale data would be required to demonstrate morbidity and mortality benefit to justify this approach over our current conventional approach to risk assessment and therapy.

CASE DISCUSSION

It is possible that the patient did not have a high grade stenosis at the time of initial stress testing. Given the high prevalence of non-stenotic coronary lesions, many if not most ACS, are caused by such lesions which are not detectable by stress testing. Our patient did well post angioplasty of the infarct-related artery and remained asymptomatic on aggressive medical therapy which included dual antiplatelet therapy, statin and beta-blocker based on

the current guidelines. While recent PRAMI data (79) suggest that our patient might have benefitted from preventive PCI to LAD lesion, there is controversy regarding the interpretation and widespread applicability of these data. When available, a validated imaging technique to assess plaque vulnerability, i.e., risk for future events, at the time of primary PCI or later on, could lead to PCI or more intensive medical therapy for the LAD lesion. Such a molecular imaging technique may also help track the effectiveness of medical therapy. In this regard, devising a global cardiac vulnerability score (80), as opposed to imaging individual plaques, might prove to be a more effective strategy.

Acknowledgments

Sources of funding:

This work was supported by National Institutes of Health R01 HL112992, R01 HL114703, and Department of Veterans Affairs Merit Award IO-BX001750.

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Abbreviations

ACS	acute coronary syndrome
CAD	coronary artery disease
CVD	cardiovascular disease
EC	endothelial cell
FDA	Food and Drug Administration
FDG	fluorodeoxyglucose
LAD	left anterior descending artery
LOX-1	lectin-like oxidized LDL receptor 1
LV	left ventricle

MI	myocardial infarction
MMP	matrix metalloproteinase
NaF	sodium fluoride
PCI	percutaneous coronary intervention
RCA	right coronary artery
RGD	arginine-glycine-aspartate
TIMP	tissue inhibitor of MMPs
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cell

ACTION ITEMS

- Enhanced basic, translational and clinical research to evaluate existing promising tracers for clinical molecular imaging
- Publicize the potential and promise of vascular molecular imaging in improving the management of patients with coronary and carotid disease
- Inter-societal co-operation to help creating a joint task force(s) to promote and complement clinical, research and educational opportunities in atherosclerosis imaging

Figure 1.

Schematic depiction of representative targets for molecular imaging of atherosclerosis and plaque vulnerability. Various aspects of plaque development and rupture have been targeted for imaging. Endothelial cell (EC) activation, subendothelial accumulation of LDL particles and monocyte recruitment are early events in atherogenesis. LDL modification (e.g., to oxidized LDL, Ox-LDL) promotes inflammation and its phagocytosis transforms monocytederived macrophages to foam cells. Activated monocytes and macrophages are highly metabolic. They express a number of chemokine receptors (e.g., CCR-2 and CCR-5) and rely on chemokine (e.g., monocyte chemoattractant protein-1, MCP-1) gradients for chemotaxis. Lectinlike oxidized LDL receptor-1 (LOX-1) is expressed by ECs, vascular smooth muscle cells (VSMCs) and macrophages. Activated macrophages produce a number of proteolytic enzymes, (e.g., matrix metalloproteinases (MMPs)) which promote inflammation, matrix remodeling, VSMC apoptosis and weakening of necrotic core. Macrophage (and VSMC) apoptosis and necrosis expose phosphatidyl serine (PS), a protein normally retained inside the cell. Impaired efferocytosis contributes to retention of apoptotic and necrotic bodies. With progressive enlargement of atherosclerotic lesions, the resultant hypoxia and release of proangiogenic factors (e.g., vascular endothelial growth factor, VEGF) triggers the development of immature intimal neovessels which in turn, contribute to inflammatory cell recruitment and necrotic core enlargement through intraplaque hemorrhage. Neovascular ECs, as well as proliferating VSMCs and activated macrophages

express $\alpha v\beta 3$ integrin. Vascular calcification is an active process mediated, at least in part, by macrophages. Inflammation, necrotic core enlargement, and thinning of fibrous cap in conjunction with perturbed biomechanics (e.g., due to foci of microcalcification) lead to plaque rupture and thrombus formation.

Figure 2.

Quantification of ^{18}F -FDG signal on ^{18}F -FDG PET/CT images of human carotid artery atherosclerosis. Various quantification methodologies have been suggested and utilized in the literature for assessment of ^{18}F -FDG uptake in atherosclerotic lesions. In this example, axial and coronal PET/CT images depict ^{18}F -FDG uptake in both carotid arteries. To quantify tracer uptake, maximum standardized uptake values (SUV) were determined in regions of interest (ROI) drawn around the artery in the axial plane along the length of the carotid artery. The “whole vessel” and the “most diseased segment” (MDS) target-to-background ratios (TBR) were calculated from the ratio of the average of the maximum SUVs and the background activity in internal jugular veins. Reprinted with permission from Tawakol et al (30).

Figure 3.

In vivo microSPECT/CT imaging of MMP activation in murine atherosclerosis using a ^{99m}Tc labeled tracer (RP805) with high affinity for activated MMPs. **(A)** Examples of CT angiography, RP805 microSPECT and fused images in a sagittal plane, demonstrating RP805 uptake in aortic arch and proximal descending aorta (red arrows) of atherosclerotic mice after 3 months of high fat diet (HFD, upper row images in panel A). RP805 uptake is markedly diminished after 1 month feeding with normal chow following 2 months of high fat diet [High fat withdrawal (HFW), lower row images in panel A]. **(B)** Examples of multi-planar reformation of in vivo CT angiography (left image in each set of images) and RP805 microSPECT (right image in each set) images of aortae demonstrating the heterogeneous

pattern of RP805 uptake throughout the aorta in HFD mice, and reduction of the signal in HFW mice or animals treated with simvastatin (Sim) or fenofibrate (Fen) for 1 month after 2 months of high fat diet to induce atherosclerosis. cpv: counts per voxel. Reprinted with permission from Razavian et al (46).

Figure 4.

¹⁸F-Galacto-RGD PET imaging in patients with internal carotid artery atherosclerosis.

Patient A (**A-D**) has a high grade stenosis of the proximal left internal carotid artery, as depicted by maximum intensity projection (MIP) magnetic resonance (MR) angiography (**A**), which demonstrates increased focal uptake of ¹⁸F-Galacto-RGD in PET (**B**) and fused PET/CT (**C**) images and strong expression αvβ3 integrin in the corresponding endarterectomy specimen (**D**). Patient B (**E-H**) has a severely stenotic atherosclerotic plaque involving the origin of the right internal carotid artery (**E**) which demonstrates minimal tracer uptake (**F** and **G**) or αvβ3 integrin expression in the endarterectomy specimen (**H**). Large arrows in A-C and E-G point to internal carotid arteries. Small arrows in B, C, F and G indicate ¹⁸F-Galacto-RGD uptake in salivary glands and pharyngeal and laryngeal mucosa. Asterisks in D and H indicate the vessel lumen. Reprinted with permission from Beer et al (64).

Figure 5.

^{18}F -Sodium fluoride (^{18}F -NaF) and ^{18}F -FDG PET imaging of coronary arteries. **Top row:** In a patient with acute ST-segment elevation myocardial infarction (A-C) proximal occlusion of the left anterior descending artery (red arrow) depicted on invasive coronary angiography (A) is associated with intense ^{18}F -NaF (B) but not ^{18}F -FDG (C) uptake in the culprit lesion in fused PET-CT images (red arrows). Yellow and blue arrows in C point to FDG uptake in the myocardium and esophagus. **Middle row:** In a patient with anterior non-ST-segment elevation myocardial infarction with (D-F), increased ^{18}F -NaF uptake is present in the culprit lesion (red arrow) in the proximal left anterior descending artery, but not in the bystander non-culprit lesion (white arrow) in the circumflex artery (E). Neither lesion has increased ^{18}F -FDG (F). Images were obtained after stenting of both lesions. **Lower row:** In a patient with stable angina (G-J) with non-stenotic atherosclerotic disease in the right coronary artery (G) normal and enhanced ^{18}F -NaF uptake is noted in the proximal (yellow

line) and mid (red line) segments of the right coronary artery, respectively (**H**). Intravascular ultrasound demonstrate features of stable plaques (fibrous and fibrofatty tissue (green), confluent calcium (white) and minimal necrosis) in the ^{18}F -NaF negative lesion (**I**) and features of plaque vulnerability [necrosis (red) and microcalcification (white)] in the ^{18}F -NaF avid lesion (**J**). Reprinted with permission from Joshi et al (73).

Table 1
Major pathological features and selected molecular targets for molecular imaging of atherosclerosis and plaque vulnerability

Process	Target mechanism	Representative tracers	Modality	Comments
Endothelial activation and lipid accumulation	Lipid accumulation	Oxidized LDL-targeted micelles (13)	MRI	Pre-clinical study High (sub-millimeter) resolution Immunogenicity and other biological effects need to be addressed Requires delayed imaging
	Adhesion molecules	VCAM-1 antibody conjugated microbubbles (14)	US	Pre-clinical study Intermediate resolution Technically challenging
Inflammation	Monocyte trafficking	^{111}In -oxine-labeled monocytes (20)	SPECT	Pre-clinical study using an FDA approved leukocyte labeling method High resolution Assesses the overall monocyte recruitment rate
		^{64}Cu labeled CCR5-targeting peptide (21)	PET	Pre-clinical study in a vascular injury model Assesses a specific aspect of monocyte recruitment
		^{64}Cu -DOTA- α MIP-II (22)	PET	Pre-clinical study in a vascular injury model Assesses expression of multiple chemokine receptors involved in monocyte recruitment
Phagocytosis		Ultrasmall superparamagnetic iron oxide (23)	MRI	Pre-clinical and clinical studies High resolution Commonly used approach, but the pathological relevance of increased phagocytosis in macrophage and plaque biology needs further investigation
		Iodinated nanoparticles (N1177) (24)	CT	Pre-clinical study High resolution but low sensitivity
Receptor expression		^{111}In and gadolinium containing LOX-1 targeted liposomes (26)	SPECT and MRI	Pre-clinical study Broad target expression Requires delayed imaging

Process	Target mechanism	Representative tracers	Modality	Comments
Matrix remodeling	Metabolism	^{18}F -FDG (27-30)	PET	Pre-clinical and clinical (mostly in carotid arteries) studies with a FDA-approved agent Myocardial uptake interferes with visualization of coronary artery uptake Limited specificity for inflammation Pathological relevance of increased glucose uptake in macrophage biology is not completely understood
		2-deoxy-2- ^{18}F [fluoro-D-mannose (38)	PET	Pre-clinical study Contribution of mannose receptor and hexose transporters to tracer uptake remains to be defined Pathological relevance of mannose uptake in plaque biology needs further evaluation
	Cathepsin activation	Cathepsin B (41) and K (40) sensitive activatable near-infrared fluorescent probes	IVFM	Pre-clinical studies Due to the limited depth of penetration of fluorescence imaging, their use is limited to invasive intravascular approach High resolution and high sensitivity
Cell death and apoptosis	MMP activation	Activatable near-infrared fluorescence probe (42)	FMT	Pre-clinical study Limited depth of penetration of fluorescence imaging, limits the application to intravascular imaging Limited selectivity of the probe High resolution and high sensitivity
		^{111}In and $^{99\text{m}}\text{Tc}$ labeled broad spectrum MMP inhibitor (43-48)	SPECT	Pre-clinical studies High affinity to activation epitope of multiple MMPs Serial imaging addressing the predictive value for progression and response to therapy
		Gadolinium conjugated broad spectrum MMP inhibitor (P947) (49, 50)	MRI	Pre-clinical studies. Moderate affinity for MMPs and several other proteases
Cell death and apoptosis	Phosphatidylserine externalization	$^{99\text{m}}\text{Tc}$ labeled annexin A5 (52)	SPECT	Preclinical and clinical studies

Process	Target mechanism	Representative tracers	Modality	Comments
Neovascularization				Externalization of phosphatidylserine externalization is not specific to apoptotic cell death
	$\alpha_v\beta_3$ integrin expression	^{18}F -labeled (61, 64) and $^{99\text{m}}\text{Tc}$ (63) labeled RGD peptides and $\alpha_v\beta_3$ -targeted paramagnetic nanoparticles (62)	PET, SPECT and MRI	Pre-clinical and clinical (PET) studies Most tracers are α_v - (not just $\alpha_v\beta_3$)-specific $\alpha_v\beta_3$ expression and activation increases in activated inflammatory cells and proliferating smooth muscle cell. Thus, RGD targeting detects angiogenesis, vascular cell proliferation and inflammation
	VEGF expression	^{89}Zr -bevacizumab (anti- VEGF-A antibody) (65)	PET	Ex vivo binding study Whether signal intensity is sufficient for imaging remains to be determined VEGF expression is not specific to angiogenesis Large size of antibodies may limit penetration into the plaques
Plaque calcification	Active calcification	^{18}F -NaF (71-73)	PET	Clinical studies in different vascular beds (aorta, carotid, coronary) FDA approved agent May be able to identify culprit lesion in ACS Low myocardial uptake reduces the interference with coronary uptake compared to ^{18}F -FDG Clinical relevance remains to be determined

FMT: Fluorescence molecular tomography
IVFM: Intravital fluorescence microscopy