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Molecular Imaging of Atherosclerosis: Clinical State-of-the-Art

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Abstract

Molecular imaging is a burgeoning field that aims to image molecular and cellular detail in living subjects. In cardiovascular research, many exciting approaches have emerged, and several are utilized in the clinic or are in the process of translation. Here, we discuss high priority clinical developments in molecular imaging of atherosclerosis, and showcase examples that may enable improved detection of high-risk plaques, clinical risk stratification, and biological assessment of pharmacotherapeutic approaches.

Keywords

Atherosclerosis; Molecular Imaging; Inflammation; PET; MRI; Fluorescence

Introduction

Physicians depend greatly on imaging techniques that help them make clinical decisions. However, when a diagnosis is made on the basis of anatomical imaging alone, the disease process has often advanced beyond the point where preventative therapy can be applied. In many cardiovascular diseases, it is vital to detect pathological and normal processes at an early, subclinical stage, to enable early and improved diagnosis, prediction and treatment (**Figure 1A**). This is particularly relevant to atherosclerosis, which can be clinically silent for decades and then manifest suddenly as an acute myocardial infarction or stroke.

The holy grail in cardiovascular prevention is to identify individuals at risk for MI or stroke. At present, structural imaging tools such as CT or intravascular ultrasound (IVUS) cannot reliably identify "vulnerable" patients with a high-risk plaque that will lead to thrombotic occlusion of a coronary or cerebral artery.[w1, w2] Our current understanding of such plaques is largely defined by post-mortem studies, but is limited by processing and only provides a single snapshot in the lifetime of a culprit lesion. These studies demonstrate that culprit lesions in acute myocardial infarction demonstrate acute plaque rupture ~60%, plaque erosion ~25%, and other mechanisms in the remainder of cases (calcified nodule, other). Plaque rupture is biologically driven by inflammatory cells (macrophages,

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lymphocytes), destabilizing proteases (matrix metalloproteinases, cathepsins), reactive oxygen species, fragile neoangiogenic vessels, and apoptotic cells that promote necrotic cores. These biological components form the foundation for a new approach to identify high-risk plaques: molecular imaging.

Molecular imaging aims to look beyond anatomy by illuminating molecules and cells in living subjects, using injectable, targeted imaging agents that can be detected and quantified by a variety of imaging systems.[w3-7] Accordingly, diagnostic and prognostic information can be obtained for specific cardiovascular diseases. This information has the potential to guide personalized medicine by optimizing the selection and dosing of disease therapies, and improve the understanding of the underlying biology of a disease. In molecular imaging, there are two components: hardware detection platforms and molecular imaging agents. Hardware platforms. Various imaging modalities, such as nuclear imaging (PET/SPECT), magnetic resonance imaging (MRI), computed tomography (CT), ultrasound (US), and optical imaging each have their strengths and drawbacks as platforms for clinical molecular imaging, including spatiotemporal resolution, depth sensing, molecular sensitivity, and availability of molecular imaging agents (**Figure 1B**). Therefore, the most advantageous imaging modality, typically a combined molecular-structural imaging platform, must be selected based on the specific disease *and* the clinical question of interest.

Molecular imaging agents

Ideal molecular imaging agents for atherosclerosis possess the following four characteristics: (1) the intended molecular target has been robustly implicated biologically, pathologically, and clinically in acute plaque syndromes; (2) the agent provides high signal-to-noise based on its targeting profile, a method of signal amplification if possible (e.g. chemical activation or biological trapping), and its pharmacokinetics (small molecules, peptides, and internalizable compounds are favorable); (3) the agent can be readily synthesized (straightforward chemistry to attach an affinity ligand to a signal-generating moiety); and (4) the imaging agent has a straightforward clinical trajectory (biocompatible, nontoxic, and inexpensive).

This review gives clinicians a broad perspective of the clinically relevant molecular imaging strategies in the field of atherosclerosis, and in particular clinical approaches. We first focus on large artery (e.g. carotid artery) applications that readily lend themselves to noninvasive imaging. Then we shift our focus towards emerging strategies for molecular imaging of coronary artery disease (CAD). Throughout the review we highlight strengths and limitations of each approach, as well as next steps and anticipated future developments.

Can we harness noninvasive molecular imaging to predict carotid plaque progression and stroke?

Carotid plaque-driven strokes cause significant mortality and morbidity, yet our current diagnostic tools cannot reliably predict which moderate lesions will progress to cause symptoms. Currently, the severity of carotid stenosis and presence of symptoms determines the indication for revascularization. In the landmark NASCET trial[w8, w9], the number-

needed-to-treat (NNT) for a symptomatic >70% stenosis was ≈ 6 , meaning that one ipsilateral stroke incident was prevented in the next 5 years by treating 6 patients. For less severe stenoses (50-69%), the NNT was ≈ 15 . Yet carotid surgery or stenting pose significant risks of death and ipsilateral stroke relative to the benefit, in particular in this latter group with 50-69% lesions. This issue is magnified for asymptomatic patients with stenotic carotid plaques.[w10]

^{18}F -fluorodeoxyglucose (FDG) imaging of glucose metabolism and inflammation in carotid arteries

^{18}F -FDG imaging is a metabolic tracer that is widely used for detection of myocardial viability and in the field of oncology. ^{18}F -FDG is a glucose analogue that enters the cell via glucose transporters, and therefore reports on glucose metabolism. After cell entry, ^{18}F -FDG is phosphorylated and is subsequently trapped within cells, allowing a concentrated ^{18}F -FDG signal to develop. As early as 2002,[1] several experimental and clinical studies demonstrated that elevated ^{18}F -FDG signal denoted metabolically active inflammatory cells, particularly macrophages, and that ^{18}F -FDG signal could be detected in atherosclerosis, aortic aneurysms and vasculitis.[w11-15] While several groups established a relationship between ^{18}F -FDG uptake and plaque macrophages, recent data also suggests a relationship between ^{18}F -FDG and hypoxia.[2][w16] ^{18}F -FDG uptake in atheroma is not associated with plaque area and thickness in some studies, but does associate several other high-risk plaque features, including positive remodeling, luminal irregularity, and low attenuation.[3]

As with all standalone molecular imaging platforms (e.g. PET, SPECT, fluorescence imaging), ^{18}F -FDG PET imaging is improved by co-registered anatomical imaging, as the molecular imaging signal can be more precisely co-localized to the tissue of interest. Anatomical imaging for PET studies is typically performed with CT (**Figure 2A**), and more recently also via MRI, as an expanding number of clinical PET/MRI systems are becoming available. A full PET/CT body scan can be performed in 1 hour, and ^{18}F -FDG is widely available, thus these factors have spurred the growth of FDG-PET in clinical atherosclerosis studies.

Key clinical studies—(1) An observational ^{18}F -FDG PET/CT study in 932 cancer patients over 29 months demonstrated that significant ^{18}F -FDG uptake in large arteries was an independent predictor of future cardiovascular events, and stronger than conventional risk factors or CT arterial calcification[4]. (2) In a smaller-sized clinical trial by Marnane et al. investigating ^{18}F -FDG uptake in 60 symptomatic patients with a recent TIA, stroke or retinal embolism, ^{18}F -FDG uptake independently predicted early stroke recurrence, *regardless* of the severity of the ipsilateral stenosis [5]. Stroke recurred in 22% percent of patients within 90 days. Of these patients, 80% had a mean above-threshold ^{18}F -FDG uptake in the carotid artery ($>2.14\text{g/ml}$, $P<0.0001$). If these exciting results are validated in multicenter trials, patients with high FDG uptake in culprit carotid arteries could then be randomized to immediate vs. usual revascularization, to determine whether recurrent stroke rates could be reduced.

Next steps for ^{18}F -FDG PET imaging of carotid atheroma: ^{18}F -FDG imaging has offered insights in natural history of plaque and inflammatory dynamics, and has established itself as a useful clinical tool to report on anti-inflammatory effects of new atheroma pharmacotherapies (discussed below).[w5, w6, w17] Prognostically, observational studies have demonstrated the potential for using ^{18}F -FDG for risk stratification.[w18, w19] However, larger prospective studies are needed to determine whether ^{18}F -FDG imaging will substantially predict the risk of stroke, and show utility beyond conventional risk factor prediction scores and carotid MRI.[w20-22] Furthermore, radiation exposure and healthcare costs of PET and co-registered CT are significant, and therefore limit PET/CT as a population-based screening tool.[w23] Therefore, even if ^{18}F -FDG PET/CT proves useful as an independent predictor of stroke, further investigation is required to understand where PET/CT will fit into screening algorithms.

^{18}F -NaF imaging of osteogenic activity

A newly described atherosclerosis clinical molecular imaging approach harnesses ^{18}F -sodium fluoride (^{18}F -NaF) PET. ^{18}F -NaF is a well-characterized PET imaging probe that identifies areas of osteogenic activity, and has been utilized for over 50 years. [w24, w25] ^{18}F -NaF allows detection of bone diseases such as Paget's disease, bone tumors, and osteoblastic metastases. Recently, ^{18}F -NaF PET imaging was applied to image osteogenic activity, or “active calcification”, in atherosclerosis. After initial feasibility studies demonstrated an association of ^{18}F -NaF with active atherosclerosis, a retrospective review of 269 cancer patients demonstrated that ^{18}F -NaF arterial signal correlated with traditional risk factors [6][w26]. In a follow-up study by the same group[w27], ^{18}F -NaF was compared to ^{18}F -FDG in 45 patients receiving both scans over a 6-month period. ^{18}F -NaF signal co-localized significantly with calcified lesions (77% vs. 15% FDG), and minimally overlapped with ^{18}F -FDG signal (6.5% lesion overlap). These findings emphasize that inflammation and active mineralization are spatially distinct.

Next steps— ^{18}F -NaF is an intriguing new biomarker for atherosclerosis that reports on osteogenic activity in atheroma. However, a substantial clinical database will be required to determine whether ^{18}F -NaF will improve risk prediction (c-statistic, net reclassification index (NRI)) beyond clinical risk scores (Framingham, Euroscore), coronary artery calcium (CAC) scores.[w28, w29] Another important question is whether the ^{18}F -NaF signal is relevant to the local pathophysiology of stroke, relevant to a systemic process associated with CV risk, or more likely, relevant to both. In addition, carotid PET/CT using ^{18}F -NaF will need to also compare favorably with MRI of carotid intraplaque hemorrhage and lipid content, useful prognostic features that can be obtained without the use of ionizing radiation or contrast agents. From a PET perspective, an overarching question remains whether ^{18}F -NaF will prove useful beyond ^{18}F -FDG – which is already established as carotid plaque inflammation imaging approach. A major advantage of ^{18}F -NaF over ^{18}F -FDG appears to be in molecular imaging of the coronary arteries (see below), and we suspect future efforts will be focused in this domain.

Additional nuclear molecular imaging agents for atherosclerosis—In a seminal feasibility study, carotid plaque apoptosis was imaged in patients using $^{99\text{m}}\text{Tc}$ -Annexin A5

SPECT.[w30] Recently, a new macrophage PET tracer (^{11}C -PK11195) also showed promise in imaging clinical carotid plaque inflammation.[w31] Expansion of molecular imaging agents will continue to drive the field forward.

Nanoparticle MRI of macrophages in atherosclerosis

Carotid MRI has markedly advanced the field of atherosclerosis imaging, and identifies plaque lipid, intraplaque hemorrhage (IPH), plaque calcification, and via gadolinium-enhanced MRI, fibrous cap thickness/integrity, as well as plaque neovasculature/permeability ($K(\text{trans})$), which is indirectly associated with inflammation and plaque instability. Carotid MRI detection of IPH in particular is consistently associated with future cerebrovascular events.[7]

Despite the substantial capabilities of structural and functional plaque MRI, specific detection of plaque inflammation was not available until the application of ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles [8][w32-35]. USPIOs are dextran-coated ~30nm diameter nanoparticles that have a strong $T2 > T1$ shortening effect, inducing signal loss on $T2$ -weighted images. USPIOs are phagocytosed by macrophages, and thus report directly on cellular inflammation within tissue. The most studied USPIO preparation for atherosclerosis is ferumoxtran-10 (Combidex in the US; Sinerem in Europe; AMAG Pharma and Guerbet, respectively). USPIO-MRI clinical carotid studies have demonstrated that asymptomatic plaques are inflamed[w36]; plaque inflammation is not related to stenosis severity[w37]; fibrous caps are thinner and more inflamed in symptomatic patients[w38]; inflammation associated with biomechanical stress[w39]; and that USPIO-MRI can report on dose responses of statin pharmacotherapy (discussed further below).

Next steps—Although promising and tested for over 10 years, ferumoxtran is not routinely clinically available, nor FDA- or EMA-approved. Another potential alternative is FDA/EMA-approved ferumoxytol, an iron replacement nanoparticle that also provides $T2$ and $T1$ contrast for MRI. However, studies have not yet demonstrated utility in atheroma targeting, possibly due to its shorter half-life ~12 hours compared to ferumoxtran-10 (~25-30 hours). A very recent development is that ferumoxtran-10 has regained life in Europe, with development rights sold to Radboud University Medical Center Nijmegen in the Netherlands.[w40] This development will also likely re-invigorate USPIO atherosclerosis studies.

Ultrasound—Carotid duplex ultrasound is routinely clinically utilized to assess stenosis and to guide carotid artery revascularization. Ultrasound-based molecular imaging approaches therefore could provide valuable molecular insights using an established clinical hardware platform. Preclinical studies have demonstrated the ability to synthesize targeted microbubbles that illuminate aspects of endothelial inflammation (e.g. cellular adhesion molecules, activated von Willebrand factor), and angiogenesis (e.g. vascular endothelial growth factor receptor), key pathways for atherosclerosis molecular imaging.[w41, w42] Translation of microbubbles into the clinical arena will galvanize this field.

Applications of noninvasive molecular imaging to evaluation of pharmacotherapies—

Plaque anatomy is an insensitive readout for many new biologically-based atherosclerosis therapeutics. Given the substantial costs of drug development, imaging approaches that can identify therapeutic winners or losers early in development can markedly streamline drug development.[w4, w5, w17, w43-45]

Statins—¹⁸F-FDG was utilized early on to assess the *in vivo* anti-inflammatory effects of statins on atheroma in large arteries.[9] In this serial PET/CT study, statin and dietary modification, but not dietary modification alone, reduced ¹⁸F-FDG carotid and aortic plaque signals (**Figure 2A**). *Dose response of statins.* A very recent 12-week intervention study by Tawakol et al. demonstrated that atorvastatin 80mg daily significantly reduced in aortic and carotid wall ¹⁸F-FDG uptake compared to a group receiving atorvastatin 10mg daily. The authors suggested that reduced ¹⁸F-FDG uptake may reflect the reduction in inflammation after higher dose statin treatment (**Figure 2B**).[10]

In a similar study that preceded the above study, the ATHEROMA study tested the dose-response of statins using serial USPIO-enhanced MRI of plaque macrophages. Serial USPIO-MRI was performed in 40 patients receiving either atorvastatin 80mg or 10mg daily treatment. The authors found that atorvastatin 80mg significantly reduced plaque inflammation after 3 months compared to baseline (−14.4%). This findings did not occur in the low dose statin group (**Figure 3**).[8] The overall results demonstrate that reduction in plaque inflammation with statins is dose-dependent. Future molecular-structural maging studies are expected to provide insights into statin-based differences in the magnitude and temporal rate of changes in plaque inflammation versus plaque volume.

CETP inhibition—The molecular and structural atherosclerosis effects of a novel cholesterylester transfer protein inhibitor, dalcetrapib, was assessed in 130 patients in a placebo-controlled multicenter study utilizing multimodality imaging [11]. This study combined multiple parameters, including ¹⁸F-FDG PET imaging, as well as MRI of plaque size, composition, and contrast-enhanced based plaque neovascularization. Dalcetrapib demonstrated no pathological effects in large arteries over a period of 24 months, a modest but non-significant reduction in ¹⁸F-FDG signal, and a significant reduction in MRI vessel enlargement after 2 years. However, the pivotal outcomes dalcetrapib trial (dal-OUTCOMES) demonstrated no cardiovascular benefit.[w46] Therefore, the value of a neutral ¹⁸F-FDG result in predicting an unsuccessful Phase III study merits further investigation.

So will molecular imaging help to predict stroke?

¹⁸F-FDG PET-based molecular imaging has advanced substantially in the last 5 years, with the recent Marnane paper intriguingly suggesting that elevated FDG signal is an independent risk factor for early recurrent stroke in symptomatic patients. To have greater impact, similar studies will need to be performed in asymptomatic patients, which will require a greater number of patients and longer follow-up, as the stroke incidence rates will be lower than in symptomatic patients. Furthermore, molecular carotid imaging combined with anatomical imaging (most likely MRI of plaque volume/IPH/lipid content) will likely help the clinician

to better predict stroke risk. Such information will be invaluable in decision-making regarding intensification of medical therapy and timing of revascularization therapies.

Can we better predict coronary artery plaque progression and myocardial infarction (MI)?

The concept of pre-identifying vulnerable coronary plaques, the underlying driver of many acute MIs and sudden cardiac deaths, has captivated cardiologists. Progress in the last ten years through advanced imaging techniques has been encouraging. However our current clinical state-of-the art approaches (e.g. PROSPECT trial [12]) do not perform well enough to serve as routine screening tools [w2]. Therefore, molecular imaging of CAD may provide a unique set of imaging biomarkers (e.g. inflammation, angiogenesis, apoptosis) that can refine risk prediction beyond structural and chemical imaging. The challenge is however much greater, given the small size and motion of the coronary arteries. Encouragingly, progress in PET/CT and intravascular near-infrared fluorescence (NIRF) are opening the window to sense coronary biology in living subjects. [w47-49]

¹⁸F-FDG PET imaging of coronary plaque inflammation

Recent reports have demonstrated ¹⁸F-FDG PET of plaque inflammation is feasible in proximal portions of the coronary beds (**Figure 4A**). To overcome the major background signal of myocardial FDG uptake, patients consumed a high-lipid, low glucose diet to decrease glucose utilization by the heart. When fused onto CT images of the coronary arteries, focal hotspots in the left main, proximal coronary arteries, and stented areas suggested that coronary inflammation could be detected and quantified.[13, 14][w50] While suggestive, limitations regarding spatial resolution (3x3x3mm voxel size), cardiac and respiratory motion, and insufficient myocardial suppression limit the capabilities of ¹⁸F-FDG PET for the coronary arteries. Attesting to these challenges, a recent study revealed that 50% of coronary segments, particularly the distal coronary bed, may be uninterpretable by ¹⁸F-FDG-PET.[15] *Next steps.* Multicenter reproducible ¹⁸F-FDG imaging protocols are needed with additional improvements in myocardial FDG suppression protocols, and possibly gating strategies for motion compensation.

¹⁸F-NaF PET imaging of coronary plaque osteogenic activity

A substantial advance in molecular imaging of coronary plaque osteogenic activity has been realized using the reporter ¹⁸F-NaF, previously validated in carotid artery subjects as discussed above. In a 119 patient substudy of an aortic valve calcification study, patients with high coronary atherosclerotic disease burdens demonstrated higher ¹⁸F-NaF activity on PET/CT (**Figure 4B**).[16] Compared to ¹⁸F-FDG, ¹⁸F-NaF did not target metabolically active myocardium, thus avoiding the issue of high background tracer in heart. ¹⁸F-NaF coronary uptake associated with older males, lower HDL levels, and the CAC score. ¹⁸F-NaF activity often localized within or adjacent to individual coronary plaques. However, 40% of patients with low ¹⁸F-NaF uptake demonstrated calcium scores above 1000, suggesting that ¹⁸F-NaF detects osteogenic activity preceding bulk calcification detected on CT. Clinically, ¹⁸F-NaF uptake was greater in subjects with a high risk factor burden, prior revascularization (38% vs. 11%), clinical diagnosis of CAD (60% vs. 26%) and prior major

cardiac events (45% vs. 23%). In addition, ^{18}F -FDG PET imaging was also performed in the same patients, and demonstrated that ^{18}F -FDG quantification was possible only in half of the coronary segments that were assessed, similar to the Saam et al. study [15]. Furthermore, unlike ^{18}F -NaF, ^{18}F -FDG did not distinguish between control patients and subjects with CAD. Very recently, a prospective clinical PET/CT trial compared coronary ^{18}F -NaF and ^{18}F -FDG uptake in patients with myocardial infarction and stable angina.[w51] In 37 out of 40 acute MI patients, greater tracer uptake was evident in culprit plaques (TBR 1.66 vs. 1.24 nonculprit plaques, $p < 0.001$). In contrast, ^{18}F -FDG imaging could not distinguish between culprit and nonculprit plaques, in part due to myocardial background uptake. In 18 out of 40 patients with stable angina, elevated ^{18}F -NaF plaque uptake was found, and these plaques had significantly greater high-risk IVUS features including greater necrotic cores, microcalcifications and positive remodeling.

Next steps— ^{18}F -NaF PET/CT may open up a new field of noninvasive coronary molecular imaging. An open question regarding risk prediction that remains is: How will osteogenic plaque imaging compare to routinely available CAC scoring? CAC has a substantially larger established clinical database, is far less expensive, and exposes the patient to less ionizing radiation. Clearly further studies are needed to establish its prognostic ability beyond risk scores and CAC. In addition, ^{18}F -NaF may be an intriguing readout for assessing the effects of new pharmaceuticals that modulate plaque osteogenesis.

Intravascular high resolution molecular imaging using near-infrared fluorescence (NIRF)

Intravascular imaging offers the potential for higher resolution and higher sensitivity compared to noninvasive techniques, albeit with the attendant greater patient risk of an invasive procedure. However, parallel to the development of intravascular structural imaging approaches for CAD, the development of high-resolution clinical molecular imaging approaches will enable a greater understanding of the role of plaque biology in driving plaque complications.[w6, w47, w49]

Intravascular NIRF imaging is a relatively new, still preclinical, approach to image biology in coronary-sized vessels in living subjects.[17, 18, 19][w6, w47, w49, w52] Compared to the visible light range, in the near-infrared (NIR) window, light travels much more efficiently through blood, and autofluorescence is lower – two features that significantly enhance the capabilities of *in vivo* fluorescence imaging. As light can be delivered efficiently through intravascular fibers suitable for human coronary arteries, akin to OFDI catheters, intravascular NIRF imaging is strongly positioned to translate to the cardiac catheterization laboratory.[20] Two promising advances in NIRF imaging include the development of a combined intravascular OFDI-NIRF catheter for integrated structural and quantitative molecular imaging (**Figure 5**) [18], and the demonstration that the FDA-approved NIRF imaging agent indocyanine green (ICG) targets plaque inflammation and lipid, via macrophage phagocytosis of albumin-ICG complexes and binding of ICG to low density lipoprotein, respectively [19]. Given the parallel development of clinical-type intravascular NIRF catheters, the availability of ICG and also new clinical NIRF agents undergoing testing in Europe (e.g. NIR fluorescent-bevacizumab for cancer angiogenesis imaging, NCT01508572), clinical intracoronary NIRF imaging trials are expected within the

next 3 years, and should enable the first high-resolution inflammation plaque imaging maps of human coronary artery disease.

So will molecular imaging improve prediction acute MI?

In comparison to molecular imaging of carotid disease and predicting stroke, imaging of high-risk CAD to predict acute MI appears significantly more challenging. This is driven by the lack of noninvasive imaging approaches that will allow the generation of large databases to study outcomes. The recent development of ^{18}F -NaF may address many limitations of ^{18}F -FDG to allow reproducible noninvasive coronary imaging, but further study is needed. Intravascular NIRF imaging is promising, but still preclinical. However, given its potential and proven integration with intravascular OCT, the ability to study inflammation safely via ICG in a OCT/NIRF PROSPECT-type natural history trial (e.g. akin to [NCT00540761](#)) could provide unique insights into plaque complications in human subjects.

Conclusions

Molecular imaging of atherosclerosis has experienced a transformative decade of translation into the clinical arena. Most applications have focused on carotid disease and aortic disease utilizing the inflammation-sensitive metabolic reporter ^{18}F -FDG PET imaging. Molecular imaging has provided valuable new insights into the role of inflammation in plaque pathophysiology and stroke, and in the assessment of atherosclerosis pharmacotherapies. In the next 3-5 years, we anticipate substantial insights into the predictive ability of ^{18}F -FDG to predict stroke beyond conventional risk factors and structural MRI. In the coronary domain, more time is needed to determine whether ^{18}F -NaF can deliver in the coronaries what ^{18}F -FDG is positioned to deliver in the carotid arteries. Finally, intravascular NIRF imaging is on the horizon and may accelerate the ability to image coronary plaque inflammation at high-resolution longitudinally. In addition to gleaning new insights into pathobiology, the field is ultimately focused on improving risk prediction to prevent the devastating complications of stroke and acute MI.

Multiple-choice questions

Question 1. Which of the following statements is NOT true:

- a. USPIO-MR imaging is the most widely used approach for molecular imaging of atherosclerosis
- b. USPIO agents are not FDA-approved
- c. USPIOs can be used to assess statin efficacy
- d. ^{18}F -NaF imaging is an agent for imaging plaque apoptosis
- e. ^{18}F -NaF imaging can be used for molecular imaging of the coronary artery tree

Question 2. ^{18}F -FDG plaque imaging is NOT useful for:

- a. Imaging carotid artery inflammation

- b. Quantification of anti-inflammatory effects of pharmacotherapeutics
- c. Imaging inflammation in aneurysms
- d. Imaging inflammation in distal coronary arteries
- e. Imaging inflammation in vasculitis

Question 3. The best application of ^{18}F -NaF imaging in atherosclerosis is for:

- a. Imaging arterial flow
- b. Imaging active mineralization
- c. Imaging inflammation
- d. Imaging lipids
- e. Imaging end-stage calcification

Question 4. Which of the following clinical imaging methods has the lowest spatial resolution?

- a. MR imaging
- b. CT imaging
- c. Ultrasound imaging
- d. PET imaging
- e. Near-infrared Fluorescence imaging

Question 5. Intravascular molecular imaging utilizes which of the following technologies to obtain high-resolution, real-time detail:

- a. MRI
- b. PET
- c. SPECT
- d. CT
- e. Near-infrared Fluorescence

Question 6. Molecular imaging is an important approach for atherosclerosis imaging for all of the following reasons EXCEPT:

- a. It offers insight into atheroma biology and pathophysiology.
- b. It offers quantitative biological assessment of anti-atherosclerotic pharmacotherapeutics.

- c. It can be performed without injection of contrast agents.
- d. It is capable of detecting a pre-disease state, offering the potential for improved risk prediction.
- e. It can be used synergistically with anatomical imaging modalities.

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Molecular imaging of atherosclerosis: Key points

- Molecular imaging technology visualizes cellular and subcellular processes in living subjects via the use of specialized, targeted imaging agents.
- Molecular imaging of atherosclerosis offers the new capabilities of identifying biologically high-risk plaques, improving risk prediction, and streamlining drug development.
- Nuclear imaging (PET, SPECT) has the greatest availability of molecular imaging agents. Agents for MRI and optical imaging are emerging.
- PET imaging with ^{18}F -fluorodeoxyglucose is a powerful and prevalent approach to quantify metabolic activity/inflammation in atherosclerosis disease. ^{18}F -fluoride is emerging as a noninvasive tracer for imaging coronary artery mineralization.
- Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles are clinical inflammation imaging agents for MRI.
- High-resolution intravascular near-infrared fluorescence (NIRF) approaches for coronary artery molecular imaging are nearing clinical trials.

Figure 1.

Clinical molecular imaging concepts. **(A)** Schematic representation of the value of molecular imaging in the detection of early disease or even pre-disease changes in patients, compared to anatomical imaging capabilities. Patient symptoms usually occur in a later phase where physiological and/or anatomical changes have occurred. **(B)** Comparative overview table of electromagnetic energy, spatiotemporal resolution of clinical systems, advantages/disadvantages of molecular imaging modalities. PET=positron emission tomography, SPECT=single photon emission computed tomography, MRI=magnetic resonance imaging, CT=computed tomography.

Figure 2.

The use of ^{18}F -FDG PET/CT imaging of inflammation in carotid artery atherosclerosis to assess anti-inflammatory effects of statins. **(A)** ^{18}F -FDG PET and merged PET/CT images of carotid artery atherosclerosis in patients undergoing dietary intervention without (1st panel) and with simvastatin (2nd panel, 3 months later) treatment. ^{18}F -FDG uptake was significantly decreased after statin treatment (white arrows), while dietary changes alone negligibly affected carotid and aortic uptake. **(B)** In a statin inflammation dose-response serial PET/CT study, 67 patients were randomized and started atorvastatin 10mg or 80mg per day. They underwent ^{18}F -FDG imaging at baseline, and after 4 and 12 weeks of treatment. After 4 weeks of statin treatment, a significant reduction ^{18}F -FDG uptake (MDS-TBR) was observed in both 10mg and 80mg groups (6.4%, 12.5% respectively). However, after 12 weeks this effect was diminished to 4.3% ($p>0.10$) in the 10mg group. In the 80mg group the effect further increased significantly to 14.4% at 12 weeks treatment. MDS-TBR=most diseased segment - target-to-background ratio. Reproduced by permission from references [9, 10].

Figure 3.

Assessment of statin anti-inflammatory effects using serial USPIO-enhanced molecular MRI of patients with carotid vascular disease. Patient underwent molecular MRI at baseline, and then started either low-dose 10mg or high-dose 80mg atorvastatin, and then underwent repeat MRI at 6 and 12 weeks. T2-weighted MR imaging of carotid artery before USPIO infusion (A) and after infusion (B) in patients receiving a low dose of atorvastatin (10mg). (A) Carotid MRI images remain similar to baseline at 6 and 12 weeks suggesting no or negligible USPIO uptake in the vessel wall from prior injections. (B) Post-injection USPIO uptake was found at all time-points (dark areas indicating MRI signal loss, yellow arrows), suggesting minimal anti-inflammatory effect of atorvastatin 10mg. (C) The 80mg atorvastatin group also demonstrated elevated USPIO plaque uptake at baseline (dark area, small yellow arrow). However, at 6 and 12 weeks after treatment, enhanced MRI signal (small blue arrows) rather than signal voids were noted, consistent with a decrease in USPIO uptake and plaque macrophages induced by high-dose statin treatment. *Reproduced by permission from reference [8].*

Figure 4.

Coronary molecular imaging using noninvasive PET/CT. (*left panels*) ^{18}F -FDG PET/CT preliminary imaging of coronary artery inflammation in subjects with CAD. (**A**) ^{18}F -FDG uptake in the left main coronary artery (LMCA) and stented lesion in a patient with an ACS. (**B**) In a patient with stable CAD, ^{18}F -FDG uptake was found in a recently stented mixed plaque in the LMCA, although to a lesser extent (**C**) modest ^{18}F -FDG uptake in a lesion that was stented months before. (**D**) ^{18}F -FDG uptake at the LMCA trifurcation in an ACS patient. The box plot depicts ^{18}F -FDG aortic uptake in ACS and stable CAD patients. The mean target-to-background ratio was higher in ACS patients as expected. (*right panels*) ^{18}F -NaF PET/CT imaging of plaque osteogenic activity in CAD patients. Two examples of patients (**A**) without and (**B**) with coronary calcification. Both patients showed lack of ^{18}F -NaF plaque osteogenic activity, consistent with absent and “burnt out” CAD, respectively. (**C**) Clear focal ^{18}F -NaF uptake in the proximal left anterior descending artery (LAD). (**D**) Increased focal ^{18}F -NaF uptake bordering calcified regions of mid-LAD. The graph plots the Framingham risk scores (FRS) in patients. Patients with elevated ^{18}F -NaF uptake had a higher FRS. Error bars denote SD of the mean. Reproduced by permission from references [14, 16].

Figure 5.

Emerging high-resolution approaches for coronary artery molecular imaging of inflammation using intravascular NIRF imaging. **(A)** Standalone intravascular NIRF molecular imaging of inflammatory cathepsin protease activity in atherosclerosis. A rabbit with atheroma was injected with a specialized NIRF cathepsin protease-activatable imaging agent, Prosense VM110, 24 hours prior to imaging. (a) Angiography of rabbit aorta, showing radiopaque tip of the imaging catheter in distal aorta, co-registered with intravascular ultrasound (IVUS) shown in a longitudinal view (b) with 2 plaque zones (P1,P2). (c) Aligned pullback image of measured NIRF signal of the catheter along the aorta (vertical axis is rotational 0-360 degrees), demonstrating increased signal in small volume plaques. (d) Fused IVUS and NIRF longitudinal map (yellow/white = strongest NIRF signal, red/black = lowest NIRF signal). High magnification (f,g) and axial IVUS images (h,i) of plaque zones P1 and P2. Scale bars, 500 μ m (a,b,c). **(B)** Hybrid, fully integrated NIRF/OFDI imaging of atherosclerosis using a single catheter. Rabbits were similarly pre-injected with Prosense VM110 24 hours beforehand. (a) OFDI image showing a plaque from 2-10 o'clock with elevated NIRF signal (yellow/white = high NIRF signal), and corresponding (b) hematoxylin/eosin (H&E) and (c) cathepsin B staining. (d) NIRF-OFDI imaging of smaller

plaques, demonstrating relatively lower NIRF signal and lower levels of (e) plaque area and (f) cathepsin B reactivity. Scale bar, 1mm. Reproduced by permission from references [17, 18]