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Imaging inflammation and neovascularization in atherosclerosis: clinical and translational molecular and structural imaging targets

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Abstract

Purpose of review—To showcase advances in molecular imaging of atheroma biology in living subjects.

Recent findings—¹⁸F-fluorodeoxyglucose (FDG) PET/CT continues to be the predominant molecular imaging approach for clinical applications, particularly in the large arterial beds. Recently there has been significant progress in imaging of neovascularization and inflammation to delineate high-risk atheroma, and to evaluate drug efficacy. In addition, new hardware detection technology and imaging agents are enabling in vivo imaging of new targets on diverse imaging platforms.

Summary—In this review, we present recent exciting developments in molecular and structural imaging of atherosclerotic plaque inflammation and neovascularization. Building upon prior studies, these advances develop key technology that will play an important role to propel new diagnostic and therapeutic strategies identifying high-risk plaque phenotypes and assessing new plaque stabilization therapies in clinical trials.

Keywords

atherosclerosis; molecular imaging; inflammation; neovascularization

Introduction

Atherosclerosis is a chronic, systemic inflammatory disease characterized by the accumulation of lipids and inflammatory cells within the artery wall [1]. In the earliest

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CONFLICTS OF INTEREST

EAO has no conflicts to declare.

stages, atherosclerosis primarily affects the intima, however, ongoing inflammation impacts deeper layers. Leaky neovessels penetrate from the adventitia, promoting atheroma expansion associated with plaque vulnerability [2]. Inflammation weakens the protective fibrous cap that can lead to plaque rupture responsible for most acute coronary syndromes. Therefore, inflammation and neovascularization are closely interrelated, and serve as key imaging and therapeutic targets to stratify high and low risk atheroma in vivo [3].

Strategies to identify inflammation and neovascularization in human atheroma rely on biology-specific, high-resolution imaging approaches. Conventional imaging can visualize structural plaque features (e.g. calcium, lipid deposits) and the degree of vessel stenosis [4]. Increasingly, molecular imaging approaches that complement structural data are unraveling key biology central to plaque stability [5]. In this review, we highlight exciting recent advances in atherosclerosis molecular and structural imaging of inflammation and neovascularization and its relationship to plaque pathobiology.

Positron Emission Tomography (PET)

FDG-PET: advances in diagnostic image quality

¹⁸F-fluorodeoxyglucose (FDG) PET/CT imaging is the most widely utilized clinical imaging strategy to evaluate plaque metabolism secondary to inflammation. FDG is a radiolabeled glucose reporter that accumulates in metabolically active cells, including atheroma. In atheroma, FDG predominately reports on plaque macrophages, although hypoxia [6•,7•], oxidized LDL [8], and other metabolically active components may contribute to plaque FDG signal. Quantitative FDG signal is generally reported as a target-to-background ratios (TBR) or standardized uptake value (SUV). *Hyperglycemia*. Elevated serum glucose competitively decreases cellular FDG uptake, thus limiting application of FDG-PET in patients with uncontrolled diabetes mellitus, a common and high-risk atherosclerosis population. Data from 195 cardiovascular disease patients demonstrated that a pre-scan glucose level >7.0 mmol/L (126 mg/dL) inversely associated with carotid and aortic wall FDG uptake [9], suggesting that FDG-PET imaging should be avoided in hyperglycemia. *Longer timepoint before FDG-PET imaging*. Delayed FDG imaging beyond 90 minutes might increase FDG bioavailability and reduce background FDG blood pool. In two studies, delaying FDG-PET imaging to 2.5–3 hours after injection significantly increased the FDG plaque SUV and TBR signal [9•,10], a simple and effective strategy to improve plaque signal-to-noise.

FDG-PET: secondary plaque inflammation readouts

Linking arterial and fat inflammation. Carotid and aortic plaque FDG activity in 173 obese patients was found to correlate significantly with FDG uptake in fatty tissue by multivariate regression analysis [11•]. Furthermore, increasing body weight associated with greater FDG signal in fat. Increased FDG metabolic activity in the spleen, a putative organ source of leukocytes and pro-inflammatory cytokines that can accelerate atherosclerosis, was associated with greater plaque FDG inflammation in 22 acute coronary syndrome patients [12••]. Remarkably, in a separate arm of this study including 464 subjects without known cardiovascular disease, splenic FDG uptake independently associated with future cardiovascular events (hazard ratio 3.3; 95% confidence interval 1.5–7.3; p=0.003) over a

median 4 year follow up period (Figure 1). The concept of targeting systemic inflammation to reduce cardiovascular events is currently being tested in multiple large clinical trials (e.g. CIRT: Cardiovascular Inflammation Reduction Trial; CANTOS: Canakinumab Anti-inflammatory Thrombosis Outcomes Study).

FDG-PET: assessment of novel plaque anti-inflammatory therapeutics

In 38 patients with familial hypercholesterolemia, arterial FDG activity modestly correlated with baseline LDL ($R=0.37$; $p=0.03$) [13•]. Following lipoprotein apheresis (>50% LDL reduction), FDG uptake significantly diminished within only 3 days, implicating high circulating lipoprotein levels as important direct mediators of chronic plaque inflammation.

A multi-center trial of 72 atherosclerosis patients on low dose statin therapy administered a new p38 mitogen-activate protein kinase (MAPK) inhibitor (BMS-582949) did not show decreased FDG plaque uptake compared to placebo [14]. As a positive control, intensification of statin therapy significantly lowered plaque FDG inflammation. These findings suggest that BMS-582949 may not be a clinically efficacious anti-inflammatory therapy for atherosclerosis. Similarly, an anti-inflammatory 5-lipoxygenase inhibitor VIA-2291 that blocks leukotriene synthesis [15], an oxidized LDL neutralizing antibody MLDL1278A to inhibit immune complex formation [16•], and an Lp-PLA2 inhibitor rilapladi that decreases bioactive inflammatory mediators [17•], all failed to meet the primary endpoints to reduce arterial FDG plaque inflammatory activity. These studies highlight the potential predictive value of FDG plaque imaging in Phase I/II that could have implications for streamlining successful phase III trials.

FDG-PET: advances in PET coronary artery imaging

Diet modulation. Coronary FDG-PET plaque imaging is limited by high background from the adjacent metabolically active myocardium. A randomized trial recently demonstrated that a high-fat, low-carbohydrate meal, followed by 12 hour fast, reliably suppressed myocardial FDG uptake in most subjects [18•]. Quality of suppression was inversely linked to circulating free fatty acid levels, which may help select patients likely to provide high-quality noninvasive FDG-PET images of coronary arteries. *Intravascular detectors.* To improve spatial resolution, intravascular nuclear scintillating balloon catheter imaging system prototypes have been engineered [19•,20]. The 1–2 μm resolution is a significant advance over the typical 3–5 mm resolution of noninvasive PET, although it remains to be seen if intravascular PET will be a practical option for clinical investigation.

New PET inflammation agents beyond FDG

¹⁸F-labeled mannose. ¹⁸F-fluoro-D-mannose (FDM) is a glucose isomer that is transported identically to FDG via glucose receptors but has a more specific targeting profile for anti-inflammatory (M2-like) macrophage populations [21••]. Compared to FDG, FDM-PET improved plaque inflammation detection capabilities with 35% greater radiotracer uptake in rabbit atheroma. The utility of FDM beyond or complementary to FDG is under investigation.

¹⁸F-sodium fluoride (NaF) PET imaging. In a major step forward in clinical molecular imaging, NaF-PET, actively used in oncology to assess cancer bone metastases, was applied to detect osteogenic activity in human coronary atheroma in vivo. NaF has minimal myocardial background compared to FDG, and therefore enables high-quality coronary PET images with good signal-to-noise. In a prospective 40 patient study with myocardial infarction or stable angina, NaF coronary plaque uptake localized to the culprit lesion in 93% of cases (TBR: 1.66 culprit vs. 1.24 non-culprit; $p < 0.0001$), and was associated with high-risk structural plaque features on intravascular ultrasound (Figure 2) [22•]. Comparatively, FDG plaque activity was not significantly different in culprit or non-culprit plaques, likely due to signal contamination from myocardial background. Prospective studies assessing NaF signal with clinical events are underway. It remains unclear if NaF will provide predictive value beyond coronary artery calcium scores, however NaF may detect a biologically more active process.

Combined inflammation and neovascularization PET imaging

Inflammation and neovascularization have been evaluated together with PET/CT via ¹⁸F-Galacto-RGD, which targets the integrin $\alpha v \beta 3$ cell membrane receptors expressed on both activated endothelial cells and inflammatory macrophages in atheroma. In 10 patients with carotid stenosis planned for endarterectomy, ¹⁸F-Galacto-RGD uptake was significantly enhanced in regions of severe stenosis compared to less stenotic zones (Figure 3), and revealed a strong relationship with $\alpha v \beta 3$ integrin expression on ex vivo analysis ($R = 0.73$, $p = 0.04$) [23•]. While correlations of ¹⁸F-Galacto-RGD with macrophages (CD68 immunostaining; $R = 0.37$) and microvessel density (CD-31 immunostaining; $R = 0.48$) were less robust, and a difference between symptomatic and asymptomatic carotid plaques could not be demonstrated in this small study, investigations in larger numbers of high-risk atheroma patients are warranted. New translatable PET agents with improved binding affinity to enhance $\alpha v \beta 3$ integrin detection, such as ⁶⁴Cu-NOTA-3-4A based on a labeled cysteine knot peptide [24], and in vivo albumin radiolabeling techniques to identify leaky neovasculature [25], are also being developed preclinically.

Single Photon Emission Computerized Tomography (SPECT)

SPECT imaging offers lower resolution than PET (10 mm vs. 5 mm), but similar high-sensitivity reporting. Typically, SPECT employs ^{99m}Tc, a radiolabel with short blood half-life and low absorbed radiation dose. *IL-2 receptor imaging.* In 10 symptomatic carotid stenosis patients, a ^{99m}Tc-labeled interleukin-2 reporter (^{99m}Tc-HYNIC-IL-2) that targets upregulated IL-2 receptor expression on plaque T-lymphocytes, was safe, readily detectable in vivo, and localized specifically on ex vivo analysis [26]. *Folate receptor- β imaging.* Human carotid endarterectomy specimens incubated with ^{99m}Tc-folate, directed at the folate receptor- β expressed on activated macrophages, suggested that ^{99m}Tc-folate detects the reparative (M2-like) subpopulation of plaque macrophages [27•], a property shared by targeted FDM-PET imaging [21]. Folate-targeted receptors have been previously reported for clinical optical fluorescence detection [28], and are also being developed for PET [29]. Validation of specific leukocyte molecular imaging markers beyond global macrophages may highlight a deeper understanding of in vivo plaque pathobiology.

Leukocyte trafficking. SPECT imaging has also been utilized to track leukocyte homing to inflamed plaques in vivo. After baseline MRI and FDG-PET/CT characterization of carotid plaque, 10 cardiovascular disease patients were administered ^{99m}Tc -labeled autologous peripheral blood mononuclear cells (PBMC) followed by serial in vivo SPECT/CT imaging over 6 hours [30•]. Compared to healthy controls, ^{99m}Tc -PBMC uptake was greater in diseased arteries (TBR 2.1 vs. 1.5; $p=0.04$). Importantly, ^{99m}Tc -PBMC showed a strong correlation ($R=0.88$; $p<0.001$) with FDG-PET metabolic activity, implying that already inflamed plaques preferentially recruit leukocytes (Figure 4). Through improved understanding of leukocyte migration from organ reservoirs, new therapies designed to mitigate inflammatory enhancement of atheroma, such as targeted delivery of plaque-stabilizing payloads coupled to labeled leukocytes, may be realized.

Vascular cell adhesion molecule (VCAM)-1. In a preclinical study, a ^{99m}Tc -labeled single domain antibody fragment (^{99m}Tc -cAbVCAM1-5) specific to VCAM-1, a surface receptor expressed on inflamed endothelium, revealed enhanced in vivo uptake in murine atheroma [31]. ^{99m}Tc -cAbVCAM1-5 plaque uptake associated with histological VCAM-1 expression, and decreased in response to atorvastatin treatment. Single domain antibody labeled agents such as ^{99m}Tc -cAbVCAM1-5 represent new molecular imaging compounds that retain antibody sensitivity and specificity in a smaller package, can be functionalized with reporters for multiple imaging platforms, and humanized for translation.

Magnetic Resonance Imaging (MRI)

Dynamic contrast-enhancement (DCE). DCE-MRI is a structural imaging modality that determines the extent and permeability of plaque neovasculature via the transfer coefficient K^{trans} , which represents movement of gadolinium into the extracellular space. In an intriguing non-invasive study assessing both neovascularization and inflammation, 32 carotid stenosis patients underwent DCE-MRI and FDG-PET/CT to measure K^{trans} and TBR, respectively [32••]. In histologically confirmed regions of high plaque inflammation and microvessels, multivariate regression modeling identified a significant link between plaque K^{trans} and TBR ($\beta=2.63$; $p<0.0001$) that was independent of the severity of anatomic stenosis or the presence of clinical symptoms. However, a separate 41 subject imaging-only study showed strong association only in symptomatic carotid stenosis patients [33•], indicating that larger sample sizes may be needed to overcome patient and plaque diversity. This work provides confirmation that plaque inflammation and neovascularization are highly intertwined in vivo, and identifies a potential population of vulnerable plaques for future outcomes studies.

Ultrasound Imaging

Vascular ultrasound is a safe plaque assessment technique with high temporal resolution, albeit tempered by relatively low spatial resolution. *Nontargeted microbubbles.* Non-targeted ultrasound contrast agents composed of gas-filled protein, lipid, or biopolymer microbubbles (MB) confined to the blood pool can identify plaque neovessels. When excited, high-frequency MB size oscillations produce a unique acoustic signal for optimized detection. In 13 patients with carotid atheroma, quantitative non-targeted MB contrast

enhancement correlated well with FDG plaque inflammation from a non-concurrent scan within 3 months ($R=0.67$, $p<0.02$), demonstrating co-localization of inflammation and neovascularization [34•].

Targeted microbubbles. To probe plaque pathology beyond structure, MB may be labeled with an antibody or small molecule that reports on a biologically-relevant process. In hypercholesterolemic primates imaged serially every 4 months for 2 years, P-selectin and VCAM-1 targeted MB identified carotid artery endothelial activation (5–7 fold enhanced MB attachment) that was in proportion to the duration of insulin resistance and prior to ultrasound-detectable plaque formation [35•]. Further preclinical evidence suggests that VCAM-1 targeted MB can serially monitor endothelial damage recovery, such as occurs after percutaneous intervention [36•]. Whether activated endothelial inflammation detected by targeted MB can be replicated in humans, is predictive of future cardiovascular events, or can be reversed prior atheroma formation, remains to be tested.

Optical Imaging

Optical coherence tomography (OCT). Light-based imaging strategies are gaining hold in clinical use due to their safety, speed and capacity for high-resolution plaque imaging. Intravascular OCT is the mainstay of current clinical optical imaging platforms, generating unsurpassed 10–20 μm resolution images of plaque structural constituents such as thrombus, thin fibrous caps, macrophages, and neovessels during rapid, automated catheter pullback at rates 40 mm/sec. In 40 patients with mild coronary disease, OCT-identified coronary plaque macrophage accumulations and vasa vasorum neovessels correlated positively with endothelial dysfunction, defined as acetylcholine-induced vasoconstriction [37•]. This study strengthens the relationship among endothelial dysfunction, inflammation, and neovascularization in atherosclerosis. Technological innovations in OCT continue, including demonstration of μOCT imaging (1 μm resolution) that can detect cholesterol crystals within the cytoplasm of plaque macrophages [38•], and the reporting of an integrated OCT and intravascular ultrasound imaging catheter [39•].

Near-infrared fluorescence (NIRF). NIRF is an evolving translational optical imaging approach utilizing targets labeled with near-infrared fluorophores [40]. NIRF is quantitative and sensitive, and can be performed through blood without flushing with excellent signal-to-noise due to the low tissue autofluorescence present at near-infrared wavelengths. In preclinical studies, NIRF imaging of atheroma inflammation with protease- or macrophage-targeted agents has demonstrated translational potential to detect high-risk plaque features. Building upon prior work [41,42], a high-speed intravascular NIRF-OCT catheter for combined molecular and structural plaque imaging was recently tested in rabbit atheroma to detect plaque inflammation using indocyanine green, a FDA-approved NIRF reporter [43•]. In an important step towards clinical translation, an integrated clinical NIRF-OCT imaging system recently approved under FDA investigational device exemption has successfully measured coronary artery plaque near-infrared autofluorescence in a first-in-human study [44•], propelling the near term prospects of a human intracoronary NIRF-OCT imaging trial. Improved automated image processing algorithms for intravascular NIRF analysis will serve to accelerate this process [45•].

CONCLUSIONS

Atherosclerosis continues to be a major cause of death and disability globally despite significant advances in detection and treatment. While structural imaging has substantially enabled this growth, new biological molecular imaging approaches will improve our understanding of atherosclerosis pathobiology and offer venues to pursue new targeted therapeutic strategies. Inflammation and neovascularization, two key high-risk atheroma features, are being investigated in vivo via an expansion of imaging strategies, and are being harnessed as imaging endpoints in trials testing novel anti-atherosclerotic treatments. Future studies will aim to demonstrate the benefit of combined molecular and structural plaque imaging in predicting adverse cardiovascular outcomes. Overall, atherosclerosis plaque imaging is growing rapidly with an exciting outlook to allow earlier detection of atherosclerosis, enable personalized therapy in patients with established disease, and improve current paradigms for cardiovascular disease treatment.

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Key Points

- * High-risk atherosclerotic plaques are characterized by chronic inflammation and leaky neovessels.
- * Molecular and structural imaging of neovascularization and inflammation are important diagnostic and therapeutic plaque imaging targets.
- * FDG-PET/CT remains a leading strategy for plaque inflammation imaging in human clinical studies.
- * Other imaging modalities including SPECT, MRI, optical, ultrasound, and MRI for high-risk plaque imaging are available.
- * New imaging agents for molecular detection of plaque inflammation and neovascularization continue to be developed.

Figure 1. FDG-PET imaging and cardiovascular outcomes in acute coronary syndrome (ACS) patients

(Top panel) FDG-PET uptake was significantly enhanced in the aortic wall (left column) and also remote hematopoietic organs (bone marrow, middle column; spleen, right column) in ACS patients over healthy control subjects. (A and B) Compared to those with low FDG uptake, ACS patients with FDG-PET activity greater than the median in the bone marrow and spleen demonstrated increased cardiovascular risk. However, after multivariate adjustment only increased FDG splenic activity remained independently associated with future cardiovascular events (hazard ratio 3.3; 95% confidence interval: 1.5 to 7.3; $p=0.003$).

FDG = ^{18}F -fluorodeoxyglucose; PET = positron emission tomography. Reproduced with permission from reference [12].

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Figure 2. NaF-PET and FDG-PET coronary atherosclerosis imaging in acute coronary syndromes and stable angina

A patient with acute ST elevation myocardial infarction demonstrating (A) angiography with proximal LAD occlusion (red arrow), (B) markedly enhanced NaF uptake at the culprit plaque (red arrow; target-to-background ratio: 2.3 culprit vs. 1.1 reference segment), and (C) no FDG activity at the culprit lesion (red arrow) despite the myocardium (yellow arrow) and esophagus (blue arrow) exhibiting strong FDG metabolism. An acute non-ST elevation myocardial infarction patient with (D) angiography identifying the LAD culprit stenosis (red arrow) and also a non-culprit stenosis (white arrow) in the left circumflex that were both

treated with percutaneous coronary intervention. After stent placement, (E) NaF was enhanced in the culprit LAD lesion (red arrow), but not the non-culprit stenosis (white arrow); (F) in contrast, FDG signal was not significantly enhanced in either lesion. A patient with stable angina revealed (G) angiography with diffuse, non-obstructive disease in the right coronary artery (red and yellow lines denote diseased regions) that on (H) NaF-PET coronary imaging showed corresponding areas of high (red line) and low (yellow line) NaF plaque activity. Radiofrequency intravascular ultrasound imaging of plaque composition revealed (I) predominantly fibrotic (green color) and calcific (white color) structure in the low NaF signal plaque, and (J) necrotic core (red color) in the high NaF signal plaque suggesting the presence of high-risk features. NaF = ^{18}F -sodium fluoride; FDG = ^{18}F -fluorodeoxyglucose; PET = positron emission tomography; LAD = left anterior descending coronary artery. Reproduced with permission from reference [22].

Figure 3. Clinical neovascularization and inflammation imaging of carotid stenosis in vivo with ^{18}F -Galacto-RGD PET imaging

Multimodality images paired with ex vivo histology from two patients (1 patient per row of images) with severe carotid artery stenosis. (A, E) Magnetic resonance angiography demonstrates high-grade stenosis of the internal carotid artery (open arrows). Despite a similar degree of stenosis, ^{18}F -Galacto-RGD PET (B) and fusion PET/CT (C) imaging shows enhanced ^{18}F -Galacto-RGD uptake in the patient in the top row (open arrow) that was (F, G) absent in the other patient. As control, both patients revealed salivary gland and pharyngeal mucosa uptake (closed arrows in images B, C, F, and G). (D, H) Immunostaining for $\alpha\text{v}\beta 3$ integrin expression demonstrated greater $\alpha\text{v}\beta 3$ presence in the patient in the top row with high ^{18}F -Galacto-RGD activity, providing histological validation for the in vivo imaging results (* = vessel lumen). PET = positron emission tomography; CT = computed tomography. Reproduced with permission from reference [23].

Figure 4. Tracking accumulation of peripheral blood mononuclear cells (PBMC) to atheroma in humans with SPECT/CT imaging

Patients with cardiovascular disease (A) or healthy controls (B) were injected with ^{99m}Tc -labeled PBMC and serial SPECT imaging 3 to 6 hours after injection performed to visualize localization of PBMC in the aorta (red). Patients with cardiovascular disease demonstrated significantly increased PBMC accumulation that correlated with FDG-PET disease severity, which was not observed in control subjects. SPECT = single-photon emission computed tomography; CT = computed tomography. Reproduced with permission from reference [30].