Imaging atherosclerosis with positron emission tomography

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Positron emission tomography (PET) provides a non-invasive method to measure biological processes that are relevant to atherosclerosis, including arterial inflammation and calcification. The vast majority of studies imaging atherosclerosis with PET have utilized the tracer 18F-fluorodeoxyglucose (FDG) to better understand how inflammation contributes to atherosclerosis development, and to test the efficacy of therapeutic interventions aimed at reducing its progression. Additional tracers such as 18F-sodium fluoride (18F-NaF) provide additional avenues for characterizing atherosclerosis development. This review examines the emerging uses of PET arterial imaging as a marker of vascular inflammation and atherosclerosis, as a prognostic tool, and as a clinical research tool. In addition, we examine emerging methods that should advance arterial imaging with PET.

Keywords Positron emission tomography • Atherosclerosis • Inflammation

Introduction

Despite significant therapeutic advances, atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death and disability worldwide. Over the past decades, non-invasive assessment of atherosclerosis has evolved considerably, from the evaluation of coronary stenosis, to more complex characterizations of plaque morphology and biological activity. These advanced imaging methods have increased our understanding of plaque biology and have also been shown to provide additional prognostic value for characterizing cardiovascular risk.

This review summarizes the current evidence for positron emission tomography (PET) as a functional marker of atherosclerosis development, and its current applications in clinical research to increase our understanding of atherosclerosis pathophysiology and treatment. We will also discuss future directions for vascular imaging using PET with novel tracers, novel PET imaging technologies (such as PET-magnetic resonance imaging systems [PET/MRI]), and future potential clinical applications.

Inflammation, atherosclerosis development, and progression

Atherosclerosis is a biologically active process where vascular risk factors promote vascular endothelial dysfunction, expression of cellular adhesion molecules, and binding of circulating inflammatory cells to the vessel wall. Monocytes transmigrate into the vessel intima, differentiate into pro-inflammatory macrophages and later engulf oxidized lipoproteins to form ‘foam cells’. Atherosclerotic macrophages secrete inflammatory cytokines and extracellular matrix molecules, which promote further accumulation of lipoproteins, monocytes, and other inflammatory cells within the expanding atheromatous lesion.

Within more advanced plaques, macrophages undergo apoptosis, and release their lipid walls and intercellular contents within the lipid core; and over time, these atheromatous lesions become increasingly hypoxic, undergo neovascularization, and develop microcalcifications. Within this environment, macrophages release proteolytic enzymes that degrade the protective fibrous cap. Accordingly,
higher-risk atherosclerotic plaques (which are most vulnerable to rupture) are characterized by relatively greater inflammation cells, a necrotic lipid core, micro-calcification, neovascularization, and a thinner fibrous cap, with inflammation playing a central role. During this process, several targets for PET molecular imaging tracers exist that can identify vascular inflammation and atherosclerosis (Figure 1).

Detecting inflammation using $^{18}$F-fluorodeoxyglucose-positron emission tomography/computed tomography imaging

The unique properties of positron emitting tracers enable their non-invasive quantification. Positron emission tomography tracers release positrons ($\beta^+\text{ particles}$) that travel 1–5 mm in tissues until they encounter an electron and produce an annihilation event. Two high-energy photons (with an energy of 511 kEv) emerge from each annihilation event, at an angle of 180°. A PET detection system detects these simultaneously arriving pairs of photons, which are registered as true events, while rejecting other detections as scatter. As a result, PET imaging has outstanding quantitative abilities, with picomolar sensitivity.

The majority of studies using PET to image atherosclerosis have utilized $^{18}$F-fluorodeoxyglucose (FDG), a glucose analogue that accumulates in cells at a rate that is directly proportional to glycolysis. $^{18}$F-fluorodeoxyglucose enters cells via glucose transporter proteins, and then phosphorylated to FDG-6-phosphate by hexokinase. However, since FDG cannot further participate in glycolysis, it becomes ‘metabolically trapped’ within the tissue in proportion to the tissue’s glycolytic rate (see Figure 2). Because phagocytes (macrophages and neutrophils) have high glycolytic rates, FDG accumulation within them is substantial. Glycolysis (and with it FDG uptake) increases in macrophages in accordance to the level of pro-inflammatory activation (e.g. macrophage TNFα production, Figure 2).$^{7,8}$ Consistent with FDG’s high accumulation in phagocytes, multiple animal and clinical studies confirm that arterial FDG uptake correlates closely with histological evidence of atherosclerotic macrophage infiltration.$^{9–11}$ Practically, FDG has regulatory approval for clinical use, and it is one of the most common cyclotron generated PET tracers, making it more available for clinical and research imaging compared with many other PET tracers.

Protocols for FDG arterial imaging typically involve administration of FDG after an overnight fast, followed by imaging 90–180 min later. PET/computed tomography (CT) imaging systems use CT co-registration to better localize arterial signals (see Figure 3).$^4$ With careful attention to the acquisition protocol, FDG measurements of the aorta and carotid arteries are highly reproducible on repeated imaging in stable patients.$^{12}$

**Figure 1** Molecular targets for positron emission tomography atherosclerosis imaging. During atherosclerosis development macrophages avidly utilize glucose, and simultaneously take up $^{18}$F-fluorodeoxyglucose. $^{18}$F-fluorodeoxymannose is also taken up by facilitative glucose transporters on macrophages, in addition to binding mannose receptors. Somatostatin receptors are also expressed on activated macrophages, and act as a target for the tracer $^{68}$Ga-DOTATATE. Macrophages-mediated inflammation can also be detected by novel tracers targeting translocator protein receptors ($^{11}$C-PK11195) and macrophage cell membranes ($^{18}$F-FMCH). Additional positron emission tomography tracers targeting atherosclerosis can identify micro-calcification ($^{18}$F-sodium fluoride), neoangiogenesis ($^{68}$Ga-NOTA-RGD, $^{18}$F-Galacto-RGD), and cellular hypoxia ($^{18}$F-FMISO). Adaptation reprinted by permission from Macmillan Publishers Ltd: Tarkin et al.,$^4$ copyright (2014).
Arterial inflammation as a predictor of plaque progression and adverse cardiovascular outcomes

In atherosclerotic lesions, arterial inflammation (by FDG-PET) corresponds with high-risk structural features, and locations with greater inflammation are more likely to manifest subsequent atherosclerosis progression (e.g. calcium deposition). In keeping, retrospective studies have shown that aortic FDG uptake predicts future cardiovascular disease (CVD) events and provides incremental predictive value above the extent of coronary artery calcium and the Framingham Risk Score (see Figure 4). Also, in a prospective study of 60 patients with recent stroke, the FDG measure of carotid inflammation independently predicted recurrent ipsilateral cerebrovascular events. Similarly, a separate study has observed an association between carotid FDG uptake and ipsilateral microembolic signals on transcranial Doppler ultrasonography (a measure of sub-clinical TIAs). While these studies are encouraging, larger prospective trials are needed to further delineate the prognostic value of arterial FDG-PET/CT imaging.

Evaluation of therapeutic interventions

Since atherosclerosis is an active inflammatory process, measuring changes in arterial inflammation can provide insight into how therapeutics agents impact atherosclerosis development. While dozens of pharmacologic agents targeting atherosclerosis have been evaluated using FDG-PET, only five drug classes have undergone evaluation with both FDG-PET/CT imaging as well as in clinical outcome trials. The first such class of drugs to undergo PET/CT...
imaging as well as endpoint trial evaluation was statins. Several groups have observed that statins provide a substantial, dose-dependent reduction in arterial FDG uptake, an observation that is consistent with their anti-inflammatory properties, as well as the dose-dependent reductions in adverse cardiovascular events seen in clinical endpoint trials.\(^{20,21}\) Similarly, in patients with impaired glucose tolerance or diabetes, Mizoguchi et al. observed that pioglitazone reduced arterial inflammation (as measured by FDG-PET/CT), a finding that is consistent with improved cardiovascular outcomes observed in clinical endpoint trials.\(^{22,23}\)

On the other hand, the absence of an effect on arterial inflammation may forecast a drug’s lack of clinical efficacy. Dalcetrapib, a cholesteryl ester transfer protein inhibitor that increases high-density lipoprotein, was associated with an improvement in one of four co-primary MRI vascular endpoints in the Dal-PLAQUE clinical study. However, dalcetrapib did not lead to improvements in any of the pre-defined FDG-PET/CT endpoints.\(^{24}\) Subsequently, in the 16 000 participant Dal-OUTCOMES study, dalcetrapib did not reduce adverse cardiovascular events compared with placebo.\(^{25}\)

Inhibition of lipoprotein-associated phospholipase A2 (Lp-PLA2), a target for reducing atherosclerosis progression, was also recently evaluated in both FDG-PET/CT and in clinical outcome studies. The FDG-PET/CT trial (of 83 individuals, studied for 3 months) showed that Lp-PLA2 inhibition failed to reduce atherosclerotic inflammation.\(^{26}\) This finding presaged the subsequently completed clinical outcome trials (of nearly 29 000 individuals), which reported the lack of clinical efficacy of darapladib.\(^{26–28}\)

Additionally, inhibitors of P38 MAP Kinase have been studied using FDG-PET imaging. In the first such study, none of the pre-defined PET endpoints were improved by the study drug (though a post hoc exploratory endpoint suggested a potential benefit).\(^{29}\) However, a subsequent study with another P38 MAP Kinase antagonist failed to demonstrate reductions in arterial inflammation on any PET/CT endpoints.\(^{30}\) Recently, a study of 3503 individuals failed...
to demonstrate a beneficial effect of P38 MAP Kinase inhibition on CVD events.  

Thus, for drugs for which there are both clinical outcomes trial data and FDG-PET imaging trial data, there is apparent concordance between imaging and clinical outcomes, though care must be taken to interpret the imaging findings in context of the pre-defined endpoints. Accordingly, relatively small and brief FDG-PET/CT imaging trials may prove useful for identifying strategies that have greater potential to succeed in reducing ASCVD endpoints in larger clinical outcome trials.

The relationship between chronic systemic inflammation and vascular inflammation

It is increasingly evident that atherosclerotic inflammation has origins outside of the arterial wall. Multi-tissue imaging with FDG-PET/CT has shown that hematopoietic tissue activation is closely linked to arterial inflammation, increased expression of pro-inflammatory leukocytes, and independently associated with an increased risk of subsequent CVD events. Moreover, multiple lines of evidence have drawn links between atherosclerotic inflammation and extra-vascular inflammatory conditions.

Synovial inflammation (measured by FDG-PET/CT) correlates with arterial inflammation in patients with rheumatoid arthritis, which may explain the increased risk of ASCVD observed in these patients. Similarly, HIV infection is associated with a higher risk of ASCVD, and in patients with HIV, FDG-PET/CT imaging demonstrates heightened arterial inflammation, which closely corresponds with markers of monocyte activation.

More common, milder forms of chronic inflammation have also been associated with atherosclerotic inflammation. Individuals with significant periodontal disease are at increased cardiovascular risk, and indeed periodontal inflammation (measured by FDG uptake) strongly correlates with histological evidence of carotid plaque macrophage accumulation (using anti-CD68 antibody staining).

Future studies will examine whether PET can be used to determine whether therapies that reduce chronic extra-vascular inflammation also decrease arterial inflammation. For example, the NIH-sponsored multi-centre TARGET Trial (Treatments Against RA and Effect on FDG-PET/CT) will test the hypothesis, in patients with rheumatoid arthritis, that more aggressive anti-inflammatory approaches (triple therapy) will have a greater impact on arterial inflammation compared with conventional therapy.

Clinical imaging of cardiovascular inflammation

$^{18}$F-Fluorodeoxyglucose-PET/CT imaging of inflammation is clinically used in the evaluation of non-atherosclerotic cardiovascular conditions. In sarcoidosis, focal myocardial FDG uptake is associated with a higher risk of adverse events, and therapeutic reductions in myocardial FDG uptake associate with improved left ventricular ejection fraction. Additionally, FDG-PET/CT imaging appears to improve diagnostic accuracy in suspected prosthetic valve endocarditis, and in the clinical evaluation of suspected implanted device infections.
At this time, there is insufficient data to employ PET imaging as a clinical tool in the evaluation of CVD risk. Moving forward, there are several avenues where PET imaging may provide incremental clinical value over current diagnostic methods. One such scenario is in patients with carotid stenosis, wherein imaging of inflammation may improve selection of patients for revascularization. This may be of particular value in asymptomatic carotid stenosis, where the risks of revascularization are not insignificant. Future studies to examine the clinical utility and cost effectiveness of such an approach are needed.

Limitations of 18F-fluorodeoxyglucose-positron emission tomography atherosclerosis imaging

Quantification of FDG uptake in the aorta or carotid arteries predict a broader range of vascular outcomes because inflammation is a diffuse process, and it is likely that heightened inflammation observed in the carotid arteries or aorta corresponds with inflammation occurring in other arteries. However, directly quantifying the 'vulnerable plaque' in smaller, coronary arteries remains a challenge for several reasons. Firstly, the spatial resolution of current PET imaging systems is limited to ~6 mm. Furthermore, significant FDG uptake can occur in the adjacent myocardium (especially under ischaemic and/or non-fasting conditions), making it more difficult to differentiate vascular inflammation from background myocardial uptake. While adequate suppression of FDG-myocardial uptake with a high-fat, low carbohydrate diet prior to PET imaging has been reported, further research is required to refine this approach. The significant cardiac and respiratory motion that occurs when imaging the coronary arteries can result in further degradation of the PET signal, and complicate assessment of the mid to distal coronary tree. Several groups are currently pursuing novel technical solutions and imaging tracers to overcome some of these challenges.

Imaging vascular calcification using 18F-sodium fluoride

To identify areas of calcification, 18F-sodium fluoride (18F-NaF) functions by exchanging fluoride ions with hydroxyl ions on hydroxyapatite crystals; and in arteries this can identify vulnerable plaques by detecting areas of active calcification and micro-calcification, where shear stress can induce surrounding microfractures and subsequent thrombosis (see Figure 1). Myocardial uptake of 18F-NaF is negligible, and early clinical studies suggest that it may be particularly well suited for coronary plaque imaging. In the coronary arteries, increased 18F-NaF uptake corresponds with a higher clinical cardiovascular risk profile and greater coronary calcium deposition on CT. In a prospective study of patients with MI also undergoing coronary angiography, higher 18F-NaF uptake was found in culprit plaques compared with non-culprit plaques (Figure 5). Additionally, in patients with stable angina, atherosclerotic lesions with higher 18F-NaF uptake are more likely to progress to MI than those with lower uptake.

Figure 5 18F-sodium fluoride uptake is increased in coronary artery culprit lesions. Joshi et al. evaluated 18F-sodium fluoride uptake, using positron emission tomography/computed tomography imaging was measured, in individuals who had a recent myocardial infarction. A red arrow marks the site of severe stenosis on the coronary angiogram for two patients (A and C). 18F-sodium fluoride imaging in those same individuals (B and D) shows intense 18F-sodium fluoride activity at the site of the culprit left anterior descending artery lesions. Group mean data (E) demonstrate that 18F-sodium fluoride activity in the culprit lesions is higher than that in non-culprit vessels. Reprinted with permission from Joshi et al.

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Novel imaging tracers and technical developments

Additional tracers in positron emission tomography-arterial imaging

Several novel PET tracers are being examined for the detection of atherosclerosis. Early clinical data suggest that arterial uptake of the PET radiotracer $^{68}$Ga-DOTATATE, which identifies arterial inflammation by targeting the up-regulated somatostatin receptor 2 in macrophages and damaged endothelial cells, correlates strongly with both coronary calcium and ASCVD risk factor burden. Additional tracers that may target activated macrophages with greater specificity include $^{11}$C-PK11195 (targeting translocator protein receptors) and $^{18}$F-FMCH (targeting macrophage cell membranes), although these have undergone limited evaluation in human studies. In a rabbit model, uptake of the FDG isomer $^{18}$F-fluorodeoxymannose has been shown to correlate strongly with macrophage infiltration in aortic atherosclerotic lesions, and was comparable with that of FDG. Neoangiogenesis is a key feature of vulnerable atheromatous lesions, and may be detected using tracers targeting integrin αvβ3 expression on activated endothelial cells (e.g. $^{68}$Ga-NOTA-RGD and $^{18}$F-Galacto-RGD), which have been shown to correspond with arterial atherosclerosis burden or arterial inflammation in animal and early human studies. Cellular hypoxia within atherosclerotic plaques can be identified using $^{18}$F-fluoromisonidazole ($^{18}$F-FMISO), which becomes reduced and binds to intracellular molecules in hypoxic conditions. In a rabbit model, increased aortic $^{18}$F-FMISO uptake was observed in animals fed an atherogenic diet compared with controls, and atherosclerotic lesions with high tracer uptake also demonstrated more histological evidence of hypoxic changes. While these additional tracers have potential to further advance PET imaging of plaque biology, additional clinical translation studies are needed to demonstrate consistent findings, and the value of these novel tracers to predict clinical vascular outcomes needs to be established.

Vascular imaging with positron emission tomography/magnetic resonance imaging

Magnetic resonance imaging (MRI) is becoming increasingly available as part of hybrid PET/MRI systems and may have additional advantages over PET/CT systems in the evaluation of vascular inflammation. Magnetic resonance imaging does not expose patients to additional ionizing radiation. Also, unlike with PET/CT, acquisition of both MRI and PET imaging can be done simultaneously, allowing for better co-registration and motion correction, which may improve coronary artery imaging. Imaging of atherosclerotic lesions using MRI can identify high-risk and prognostically significant anatomic features of vulnerable plaques (e.g. fibrous cap thickness, and the presence of a necrotic core, intra-plaque haemorrhage, or mural thrombus) that may be complementary to biological data obtained through PET. Although few clinical studies have formally compared PET/MRI with PET/CT systems for characterizing vascular inflammation, results thus far suggest that the FDG-signal is similar and well correlated between modalities.

Conclusions

Significant advances have been made in our understanding of inflammation and its impact on atherosclerosis, and PET has emerged as a predominant non-invasive imaging tool for characterizing arterial inflammation. Its use has increased our understanding of arterial inflammation associated with chronic inflammation, and guided the development of treatments that aim to reduce vascular inflammation to improve upon cardiovascular outcomes. It can provide important prognostic information related to vascular biology, and future studies are needed to examine its clinical role in improving risk stratification and directing therapies. Further innovations in PET molecular imaging with novel tracers and improved imaging techniques will continue to advance our understanding of atherosclerosis development.

Authors’ contributions

P.J. and A.T. drafted the manuscript and made critical revision of the manuscript for key intellectual content.

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