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Review article

New methods to image unstable atherosclerotic plaques

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ABSTRACT

Atherosclerotic plaque rupture is the primary mechanism responsible for myocardial infarction and stroke, the top two killers worldwide. Despite being potentially fatal, the ubiquitous prevalence of atherosclerosis amongst the middle aged and elderly renders individual events relatively rare. This makes the accurate prediction of MI and stroke challenging. Advances in imaging techniques now allow detailed assessments of plaque morphology and disease activity. Both CT and MR can identify certain unstable plaque characteristics thought to be associated with an increased risk of rupture and events. PET imaging allows the activity of distinct pathological processes associated with atherosclerosis to be measured, differentiating patients with inactive and active disease states. Hybrid integration of PET with CT or MR now allows for an accurate assessment of not only plaque burden and morphology but plaque biology too. In this review, we discuss how these advanced imaging techniques hold promise in redefining our understanding of stable and unstable coronary artery disease beyond symptomatic status, and how they may refine patient risk-prediction and the rationing of expensive novel therapies.

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1. Introduction

Atherosclerotic plaque rupture is the primary mechanism responsible for two of the biggest killers worldwide: myocardial infarction and stroke [1]. In 2015, 423 million people were estimated to be living with cardiovascular disease, and it caused an estimated 18 million deaths. Whilst the clinical effects of atherosclerotic plaque rupture can be devastating, the development of atheromatous plaque is itself a silent and for many a benign process. Indeed, atherosclerosis is an almost ubiquitous finding in older patients the majority of whom will never suffer a cardiovascular event. Perhaps, the major challenge facing contemporary cardiovascular researchers is therefore to develop methods of accurate risk prediction without over medicalizing the population as a whole. In this review, we will briefly discuss the pathophysiology of atherosclerosis before investigating novel non-invasive imaging methods aimed at detecting unstable atherosclerotic plaque and measuring disease activity in the coronary arteries and large vessels. These advanced imaging techniques hold promise in redefining our understanding of stable and unstable coronary artery

disease beyond a patient's symptomatic status with the potential to improve our pathological understanding, to refine patient risk-prediction and to appropriately target expensive novel therapies.

1.1. Pathophysiology of atherosclerosis

Atherosclerosis is a smouldering immunoinflammatory disease fuelled by lipids [2]. It is characterised by focal thickening of the arterial intima (plaque formation) in medium and large sized arteries. Within the plaques lipid, inflammatory infiltrates, smooth muscle cells and connective tissues are found. An injury to the plaque cap known as a plaque rupture results in exposure of its core contents to the blood, causing acute thrombus formation and either partial or complete occlusion of the vessel lumen [3]. Atherothrombosis from plaque rupture is the most common cause of fatal myocardial infarction, accounting for approximately three quarters of cases, with plaque erosion accounting for the remaining quarter [4]. However, the majority of coronary plaque rupture events appear to be clinically silent, resulting in plaque growth rather than myocardial infarction.

Atherosclerosis begins in a hypercholesterolaemic state where low-density lipoproteins (LDL) infiltrate the endothelial wall. Subsequent oxidation of LDL molecules causes an inflammatory response with infiltration of T-lymphocytes and macrophages that consume LDL and form foam cells. This is initially protective, but

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with further LDL accumulation, macrophage cell death is ultimately triggered, contributing to further inflammation and the development of a necrotic core of soft unstable atheroma. Plaque inflammation also triggers smooth muscle cell loss and the production of matrix metalloproteinases (MMP) that weaken the fibrous cap, predisposing it to plaque rupture [2,5]. The thick necrotic acellular lipid core becomes increasingly hypoxic, stimulating angiogenesis, with the formation of immature microvessels prone to intra-plaque haemorrhage (IPH) [6–8].

Similar to tuberculosis [9], calcification of atherosclerotic plaque is thought to be a healing response to intense necrotic plaque inflammation characterised by two distinct stages. In the latter stage of macrocalcification, the healing process is complete and the plaque stabilised [10–12]. By contrast, the earlier stage of microcalcification is a common feature of ruptured and unstable plaques where healing is incomplete, inflammation remains active and the fibrous cap weakened by the tiny calcific deposits [13–15].

Unstable plaques at risk of rupture, therefore, have certain pathological features, including a large necrotic core, thin fibrous cap, inflammation, hypoxia, haemorrhage and microcalcification. By contrast, stable plaques at low risk of rupture have different characteristics, including a thick fibrous cap and macroscopic calcification. Advanced imaging now allows us to identify these plaque characteristics *in vivo* and determine whether patients predominantly have stable or unstable atheroma. Development of hybrid molecular imaging allows us to measure disease activity in the coronary arteries, directly. These developments hold promise in altering how we define stable and unstable atherosclerosis and in refining risk prediction beyond standard approaches. However, it should be noted that the plaque characteristics and pathophysiology underlying plaque erosion remain poorly understood, representing an important limitation of this approach.

1.2. Atherosclerotic plaque imaging

Direct imaging of coronary atherosclerotic plaque is now possible with CT calcium scoring (CACs) and coronary computerised tomography angiography (CCTA). This has permitted more accurate determination of coronary plaque burden, the presence of both obstructive and non-obstructive disease and assessments of plaque composition. Magnetic resonance (MR) whilst not as advanced as CT can provide similar information without radiation exposure, whilst novel PET approaches allow, for the first time, assessment of coronary disease activity. These plaque-imaging techniques are developing rapidly and, in the case of CT, starting to enter routine clinical practice. They are discussed in greater detail below and illustrated in Fig. 1.

1.3. Measures of plaque burden

Atherosclerotic plaque burden can be measured in different vascular beds using multiple different modalities, including ultrasound, CT and MR. Regardless of the methodology, plaque burden assessments provide powerful prognostic information, based upon the rationale that the greater number of plaques, the more likely a plaque is to rupture and cause an event.

CT calcium scoring is the best studied technique having been incorporated in to clinical guidelines [16] and providing prognostic information of incremental value to standard risk factor assessments [17]. However, most patients with high CT calcium scores will never suffer a clinical event. This may be because whilst CT calcium provides a surrogate of global plaque volume, it actually targets stable macrocalcific plaques, not the unstable plaques at highest risk of rupture. Moreover, CT calcium scoring cannot differentiate stable burnt-out disease from active unstable

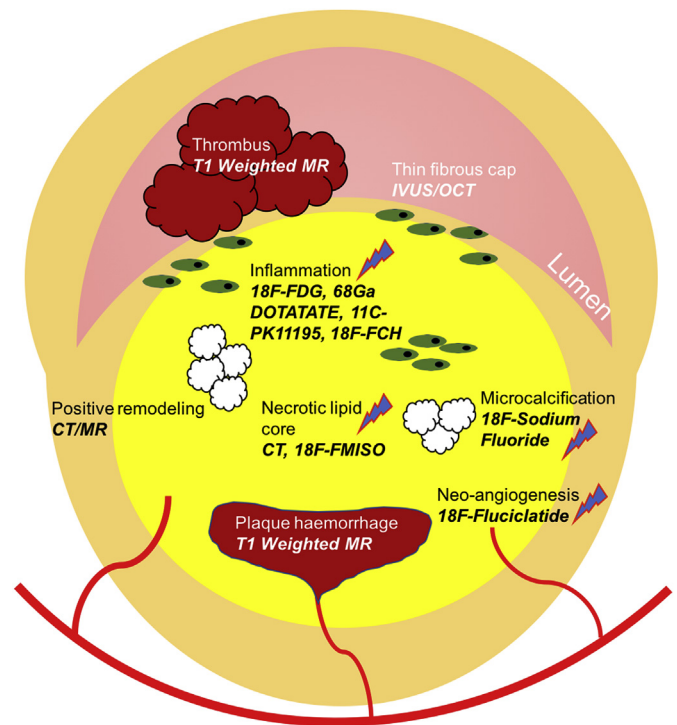


Fig. 1. Schematic representation of morphological and biological targets for unstable plaque imaging.

atheroma. Methods that can directly quantify unstable plaque and assess disease activity are therefore required.

1.4. Plaque morphological characteristics

Culprit plaques that have ruptured and caused an event have certain histological characteristics. Indeed, many retrospective and pathological studies have demonstrated the thin capped, fibro-fatty atheromatous (TCFA) plaque as the cause for the majority of myocardial infarctions and strokes [15,18–20]. Other recognised features of potentially unstable plaques are microcalcification, positive remodelling, inflammation and plaque haemorrhage [21], each representing a potential imaging target to improve the identification of high risk patients. As with plaque burden, multiple imaging modalities have been employed to better characterise plaque morphology.

IVUS can assess plaque burden, positive remodelling and lipid core. Moreover, virtual histology IVUS (VH-IVUS) allows direct detection of the VH-IVUS TCFA in the coronary vasculature [22–24]. However, in the PROSPECT study of the 695 patients, whilst 595 VH-IVUS TCFA were identified, only 6 MIs were observed over a 3-year period [25]. Comparable findings were reported in the VIVA study [26], suggesting low predictive value of these supposedly high-risk plaques [27,28]. We, therefore, prefer the term unstable plaque characteristics.

The other main invasive assessment of unstable plaque is OCT. This technique is particularly good at imaging the fibrous plaque, with the ability to identify thin caps and both plaque rupture and erosion [29,30]. Emerging OCT techniques offer assessment of further unstable plaque characteristics including plaque macrophages and angiogenesis [31–33]. However, similar to VH-IVUS, OCT-defined unstable plaques only rarely cause clinical events, so that plaque directed therapies cannot be recommended [34].

Contrast coronary CT angiography (CCTA) offers major

advantages compared to CT calcium scoring. First, it can identify obstructive stenosis [35], providing improved diagnostic accuracy and clinical outcomes in the assessment of patients presenting with chest pain [36,37]. Second, it can inform about plaque morphology. Unlike CACs, CCTA can identify non-calcified as well as calcified plaque and can identify multiple unstable plaque characteristics. These include low attenuation as a marker of necrotic core, positive remodelling and spotty calcification (early macrocalcific deposits). Multiple studies have demonstrated these features in culprit plaque post-myocardial infarction [38–41]. Moreover, Motoyama recently demonstrated that patients with these unstable CT plaques are at an elevated risk of subsequent myocardial infarction [42].

MR coronary angiography is a developing technology that remains inferior to CCTA. However, MR can also identify unstable coronary plaque characteristics, with T1 weighted imaging holding particular promise. This approach makes use of the high T1 signal associated with methaemoglobin, a key constituent of fresh thrombus. On this basis, T1-weighted imaging can identify both intraplaque haemorrhage and intraluminal thrombosis [43,44], with increased signal localising to culprit carotid and coronary plaques. Moreover, increased carotid and coronary signal identifies patients at increased risk of subsequent stroke and MI, respectively [45–47].

Several common themes appear to be emerging across these vulnerable plaque studies. The first is that in prospective studies, so-called vulnerable plaques in fact only rarely go on themselves to cause clinical events. The majority will likely heal without consequence whilst others may rupture sub-clinically without prompting myocardial infarction. Only a tiny minority will go on to cause a clinical event. In these circumstances, invasive imaging and therapies aimed at individual plaques do not make sense. However, the CT and MRI studies discussed above suggest that unstable plaque detection still holds promise in improving risk stratification at the patient level and in directing the use of systemic therapies. Unstable plaques rarely exist in isolation and their identification can highlight patients with an active disease process and an on-going propensity to develop plaques with an unstable phenotypes [48]. Whilst most of the individual plaques will heal, with on-going formation there is an increased probability of one such unstable plaque eventually rupturing and causing an MI. Further work is required to demonstrate the clinical utility of advanced coronary plaque characterisation. In particular, it remains unclear whether unstable plaque identification can improve patient risk stratification over and above more simple assessments of plaque burden.

1.5. *Imaging disease activity in atherosclerosis*

The emergence of hybrid PET/CT scanners with advanced imaging capability has for the first time allowed assessment of atherosclerotic disease activity. In principle, the activity of any disease process can be assessed, however, in practice this is dependent on the availability of PET tracers. To date, vascular PET imaging has focused upon 18F-FDG: a marker of glucose utilisation and a non-specific marker of inflammation that is limited to imaging of the carotid arteries, femoral arteries and aorta. However, novel tracers are quickly emerging specific to multiple different pathological processes. Moreover, technological developments aimed at improving the (currently limited) spatial resolution of PET now allow quantification of certain tracers in the coronary arteries. Below, we discuss the current status of atherosclerotic plaque imaging using PET, SPECT, and a variety of different tracers.

1.6. *Imaging inflammation with 18F-fluorodeoxyglucose (FDG)*

As described above, atherosclerosis is a chronic inflammatory

disease characterised by lipid deposition and macrophage infiltration. The recent CANTOS RCT of 10,061 patients with previous MI and elevated CRP levels demonstrated, for the first time, that an anti-inflammatory agent (150 mg Canakinumab a monoclonal antibody targeting Interleukin 1 β) can reduce hard clinical end points when compared to placebo. This trial therefore confirmed the close relationship between inflammation and cardiovascular events independent of lipid levels [49], and established inflammation as a key target for therapeutic and novel molecular imaging approaches.

18F-FDG PET has in fact been used to image inflammation for over a decade, based upon the high glucose consumption of vascular macrophages [50]. 18F-FDG competes physiologically with glucose to enter the cell, where it becomes trapped and unable to undergo further metabolism. FDG, therefore, accumulates in metabolically active cells at a rate proportional to their glycolytic activity [51].

The association between 18F-FDG activity and unstable atherosclerotic plaque was first demonstrated by Rudd et al., who observed increased uptake in symptomatic carotid plaques ipsilateral to a recent stroke compared to the asymptomatic contralateral plaque. Autoradiography of excised plaques confirmed greatest uptake in macrophage rich plaques [52]. Here was the first human evidence that active inflammation assessed by FDG PET could accurately identify clinical plaque rupture. Other studies have confirmed the close association between FDG uptake and macrophage burden on histology [53] as well as upregulated gene expression of CD68 (a macrophage specific marker) [54]. Association with other markers of plaque vulnerability have also been established, including circulating MMP-1 levels [55], whilst an elegant mechanistic study demonstrated that hypoxia potentiates 18F-FDG uptake by macrophages [56].

Recent studies have explored vascular 18F-FDG activity in systemic inflammatory conditions, providing mechanism for the increased cardiovascular event rates observed in these patients. In particular patients with psoriasis have demonstrated increased vascular 18F-FDG uptake beyond that explained by their cardiovascular risk factors [57], with similar results described for subjects with both HIV and rheumatoid arthritis [58,59]. Moreover, Tahara et al. reported increased carotid 18F-FDG uptake in patients with metabolic syndrome [60], whilst Kim described increased activity in diabetics and those with impaired glucose tolerance independent of blood glucose [61].

Tawakol et al. recently elucidated the link between emotional stress, increased vascular inflammation and cardiovascular events. This elegant study embraced FDG's lack of specificity, indeed, it harnessed it to investigate the relationship between processes occurring in disparate organ system. The authors used resting 18F-FDG uptake in the amygdala as a marker of emotional stress and demonstrated an association with both FDG uptake in the bone marrow (as a marker of hemopoietic activation) and the vasculature (as a marker of vascular inflammation). They also demonstrated that increased 18F-FDG uptake in these tissues identified patients with an increased risk of cardiovascular events, proposing that stress caused macrophage mobilisation from the bone marrow, increased vascular inflammation and clinical events [62]. Other retrospective studies have also linked increased vascular 18F-FDG activity to subsequent cardiovascular events. In a study of over 500 individuals devoid of a previous history of cardiovascular disease, uptake in the ascending aorta strongly predicted the development of cardiovascular disease independent of conventional risk factors [63]. However, prospective outcome studies in non-biased patient populations are now required to confirm the prognostic capability of 18F-FDG PET.

18F-FDG PET is emerging as a novel method for assessing the

anti-inflammatory effects of both established and novel atherosclerotic medication. The clinical efficacy of statins is well established [64], it is, therefore, reassuring that statins consistently reduce the arterial FDG signal in a dose dependent manner [64–66]. Similarly, pioglitazone, which is associated with lower rates of adverse cardiovascular outcomes in diabetics [67], has been shown to attenuate vascular 18F-FDG uptake, further supporting links between diabetes and vascular inflammation independent of blood glucose levels [68,69].

By comparison, Dalcetrapib, a CETP inhibitor which increases high density lipoprotein (HDL) levels, did not have any effect of vascular 18F-FDG activity; an effect that was mirrored by a similar inability to reduce adverse cardiovascular events [70,71]. Similarly, the lipoprotein-associated phospholipase A2 (implicated in atherosclerosis progression) inhibitor Rilapladib failed to reduce both 18F-FDG uptake [72] and cardiovascular events in the STABILITY trial of 15,000 patients [73]. Finally, the P38 mitogen activated protein kinase (MAP) failed to have a positive impact on either the arterial 18F-FDG activity [74,75] or cardiovascular events [76].

To summarise, large vessel FDG PET is a marker of vascular inflammation, whose uptake acts a surrogate of plaque macrophage burden and is associated with conventional cardiovascular risk factors and systemic inflammatory conditions. Importantly, FDG uptake can be attenuated with medication, leading to its adoption as an endpoint in trials assessing the anti-inflammatory effects of novel therapies. With the development of increasingly expensive atherosclerosis drugs, FDG may also prove useful for identifying patients most likely to gain clinical benefit, although prospective outcome trials are first required to assess whether it provides independent prognostic information.

Whilst FDG's lack of specificity has allowed investigators to assess the links between vascular inflammation and both the brain and bone marrow, it has limited the application of 18F-FDG PET to the coronary arteries. Indeed, physiological uptake of 18F-FDG by the myocardium means that coronary interpretation is challenging, even despite meticulous dietary preparations [77]. The desire to assess disease activity in the coronary arteries, the vascular bed responsible for the majority of clinical events, has prompted investigation of multiple novel tracers discussed below [78] (Fig. 2).

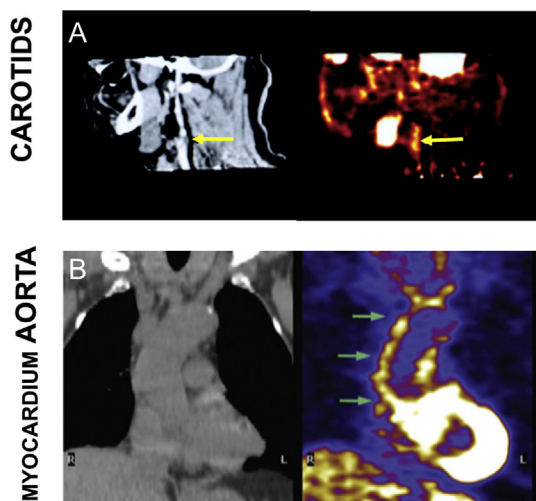
1.7. Other PET tracers targeting inflammation

1.7.1. 68 Gallium-dotatate

Gallium 68 labelled DOTATATE targets the somatostatin receptor subtype 2 (SSTR2) found on the surface of macrophages [79]. Pre-clinical studies have confirmed the superiority of 68Ga-DOTATATE over FDG in terms of macrophage specificity in atherosclerotic inflammation [80] [81]. Early retrospective clinical studies in cancer patients supported the pre-clinical findings and interestingly found some discordance between FDG and DOTATATE activity provoking further research [81–83]. Recently, a prospective clinical study pitched DOTATATE versus FDG in the setting of established aortic, carotid and coronary atherosclerosis. DOTATATE demonstrated increased uptake in culprit coronary and carotid arteries and outperformed FDG in discriminating between unstable and stable coronary plaque as defined by CT. Histological validation confirmed that DOTATATE uptake occurred in CD68-positive, macrophage-rich carotid plaques. Specific binding to pro-inflammatory M1 macrophages was demonstrated through exclusive expression of the SSTR2 receptor [78]. These findings poise DOTATATE PET as an exciting alternative to FDG, providing more specific information about M1 macrophages in atherosclerotic plaque that can be translated in the coronary arteries. Further studies are on going in this area (Fig. 3).

1.7.2. 18F-fluorocholine (18F-FCH)

Like many inflammatory tracers, 18F-FCH was first developed for use in oncology. Via specific transport mechanisms, choline is taken up into the cell, phosphorylated and metabolized to phosphatidylcholine and eventually incorporated into the cell membrane. Increased choline uptake has been shown in tumor cells and activated macrophages [84]. Its potential for human atherosclerotic imaging was confirmed by *ex vivo* and *in vivo* mouse model studies of atherosclerosis where uptake was significantly higher in atherosclerotic versus healthy aorta [85–87]. A strong association between large vessel uptake and atherosclerotic changes in the arterial wall has been demonstrated in a cohort of prostate cancer patients with an apparent inverse relationship with calcification [88]. This inverse relationship was confirmed in a larger study of 93 prostate cancer patients where only 6% of tracer uptake co-localised with macrocalcification calcification and in all calcified



	¹⁸ F-FDG study (+)	¹⁸ F-FDG study (-)
Clinical Outcome (+)	Atorvastatin	
Clinical Outcome (-)		Dalcetrapib VIA-2291 Losmapimod oxLDL antibody

Tawakol A et al, JACC 2013;62(10):909-17.
 Fayad ZA et al, Lancet 2011;378(9820):1547-59.
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 Lehrer-Graiwer J et al, JACC CI 2015;8(4):493-4.

Fig. 2. FDG performance in early phase clinical trials. (A) CT on the left with corresponding PET image on the right demonstrating FDG uptake in culprit carotid plaque. (B) How FDG PET CT imaging can be applied to the aorta and myocardium.

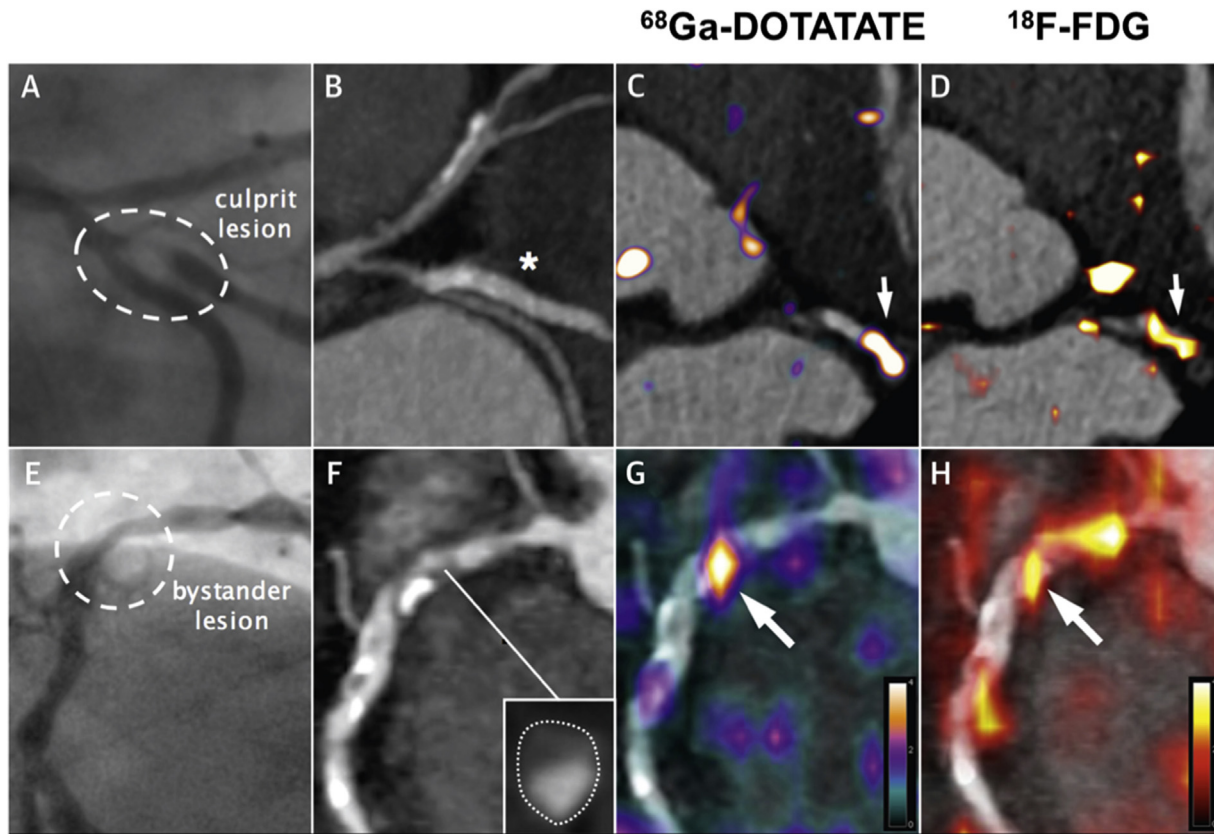


Fig. 3. Detection of coronary atherosclerotic inflammation by ^{68}Ga DOTATATE.

The panels in the top row show the culprit lesion in the circumflex artery with corresponding angiogram (A), CT (B), and PET CT with ^{68}Ga DOTATATE (C) and FDG (D). The bottom row of panels illustrates a bystander lesion in the proximal right coronary artery which is appears of moderate stenosis on angiography (A), appears non-calcified and positively remodeled on CT angiography (F) and has a clear correlation of DOTATATE (G) and FDG (H) uptake [78].

lesions only 1% had tracer uptake [89]. Further prospective research is now required.

1.7.3. ^{11}C -PK11195

^{11}C -PK11195 is a ligand of the 18-kDa translocator protein (TSPO), which is highly expressed in human macrophages [90]. It has been most widely used for neuroinflammatory imaging owing to high microglial and low neuronal uptake and was first explored in the cardiovascular system in patients with large vessel vasculitis [91]. In atherosclerosis, ^{11}C -PK11195 demonstrates increased uptake in culprit carotid plaques following stroke, and a close association with inflammatory cell burden in carotid endarterectomy specimens [92]. However, significant challenges with data interpretation remain due to genotypic differences that govern the metabolism of this tracer and have a profound effect on tracer activity. Moreover, the short half-life (20 min) of the tracer necessitates an on-site cyclotron, limiting its availability and use. Other next generation TSPO tracers are in development that seek to use ^{18}F as a more convenient radiolabel and to avoid the genetic influences on metabolism.

1.8. Other novel atherosclerotic tracers

1.8.1. ^{18}F -fluoromisonidazole (^{18}F -FMISO)-hypoxia

FMISO targets hypoxia, an important process linked to atherosclerotic progression. As a plaque grows its core becomes progressively hypoxic promoting inflammatory cell infiltration and further oxygen consumption, thereby further worsening hypoxic conditions. Micro vessel formation ensues and is the necessary

substrate for intraplaque haemorrhage; a key marker of plaque vulnerability. In a rabbit model of aortic atherosclerosis, ^{18}F -FMISO was compared with ^{18}F -FDG. *In vivo* and *ex vivo* PET imaging demonstrated strong accumulation of ^{18}F -FMISO accumulation in areas of aortic atheroma, co-localising to regions of hypoxia detected on immunohistochemistry with pimonidazole [93].

A recent study by Joshi et al. (Fig. 4) investigated FMISO PET in patients with recent TIA or stroke ($n = 16$). FMISO uptake was slightly higher in symptomatic plaques than contralateral lesions (TBR 1.11 ± 0.07 vs. 1.05 ± 0.06 ; $p < 0.05$) and demonstrated a correlation with FDG activity, suggesting once again that hypoxia contributes to the inflammatory response and FDG uptake [78,94]. This tracer appears to hold future promise for atherosclerotic plaque imaging promoting greater understanding of the relationship between hypoxia and inflammation.

1.8.2. ^{18}F -fluciclatide-angiogenesis

^{18}F -fluciclatide is a novel $\alpha_v\beta_3$ selective radiotracer and has been investigated as a marker of angiogenesis following MI with encouraging results. In a study of 37 patients, uptake was significantly increased at sites of recent MI thereby acting as a biomarker of cardiac repair and importantly predicting regions most likely to recover contractile function [95]. Data regarding arterial uptake of this tracer has not yet been published.

1.8.3. Annexin V (SPECT tracer) – apoptosis

$^{99\text{m}}\text{Tc}$ labelled Annexin V has a high affinity for phosphatidylserine which is predominantly found on the plasma membrane of apoptotic cells. It has primarily been used in oncology but has

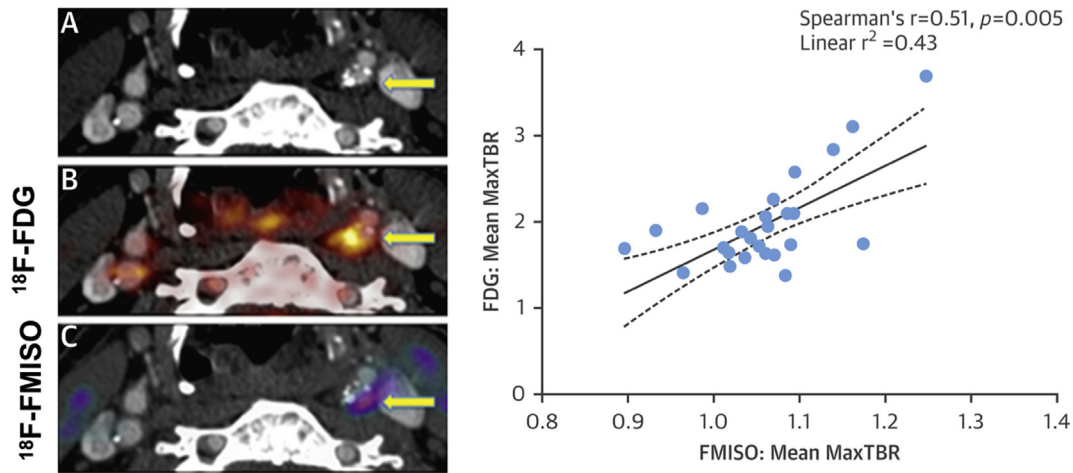


Fig. 4. FMISO quantifies hypoxia in carotid plaques.

(A) Plaque in left internal carotid on contrast enhanced CT. Co-registered PET images with FDG (B) and FMISO (C) demonstrate a strong correlation of tracer uptake.

also been studied in heart failure, cardiac transplant recipients and atherosclerosis [96]. In a small study of those undergoing carotid endarterectomy, uptake was shown to strongly correlate with high risk plaque characteristics (macrophage infiltration and intraplaque haemorrhage) suggesting a potential role for unstable plaque imaging [97].

1.8.4. 18F-sodium fluoride-microcalcification

The PET tracer 18F-sodium fluoride (18F-NaF) has been used for many decades as a maker of increased bone metabolism. It works via the exchange of 18F-NaF with hydroxyl groups on hydroxyapatite: a key structural component of both bone and vascular calcification [98]. More recently, 18F-fluoride has been used to investigate developing microcalcification in the vasculature, emerging as a promising new PET radiotracer in the field of cardiovascular medicine.

The intimal calcification observed in atherosclerosis is thought to occur as a healing response to intense necrotic inflammation within the plaque. At the outset, small crystals of hydroxyapatite begin to coalesce into organised areas of microcalcification. The natural continuation of this process is the formation of dense sheets of macroscopic calcification that stabilize the plaque, effectively walling off the necrotic core from the lumen. Whilst the end-stages of macroscopic calcification impart stability, the early stages of microcalcification are instead consistently associated with culprit and unstable plaque phenotypes and an increased propensity to rupture. In part, this may reflect the ongoing plaque inflammation that is yet to heal and in part the increased mechanical stress caused by microcalcific deposits in the fibrous cap that weaken its tensile strength and predispose it to rupture [13].

Whilst CT is able to detect macroscopic calcification, including early spotty calcification it is unable to resolve microcalcification. However, this has now become possible with 18F-fluoride PET. Irkle et al. demonstrated that 18F-fluoride binds preferentially to regions of developing microcalcification in carotid atheroma, with little or no binding to non-calcific tissue types [99]. Certainly, 18F-fluoride PET provides different information to CT. In carotid atheroma, coronary atheroma, aortic atheroma, aortic stenosis and AAA, 18F-fluoride has consistently demonstrated a different pattern of uptake to the macroscopic calcium observed on CT [98,100–102]. In aortic stenosis, areas of increased 18F-fluoride activity predict where novel deposits of macroscopic calcium will deposit, providing excellent prediction of progression in CT calcium scores

of the valve [103,104]. Similar results have also been described in atheroma, with a recent prospective study demonstrating the ability of 18F-fluoride PET to predict progression in the CT calcium score over 1 year in 34 patients [105]. This is potentially important as fast calcium score progression is known to offer powerful prognostic information of incremental value to single baseline measurements [106].

Dweck et al. first demonstrated that coronary 18F-fluoride uptake was associated with cardiovascular risk, demonstrating significant associations between coronary arterial NaF uptake and prior coronary events, angina status and Framingham risk scores ($p = 0.016$, $p = 0.023$ and $p = 0.011$, respectively) [96]. This was supported by a study of 89 healthy adults, in whom 10-year risk

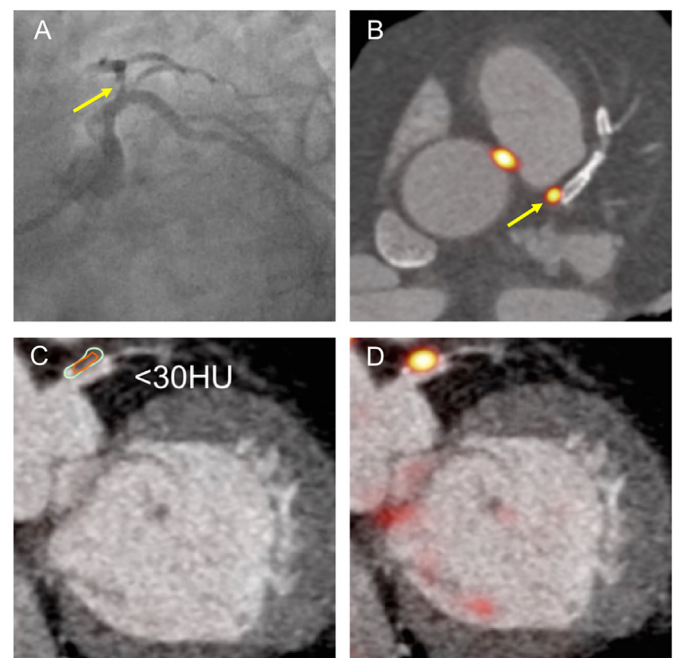


Fig. 5. CT characteristics of ^{18}F -NaF uptake.

Coronary angiography in LAO caudal view showing severe proximal LAD lesion (A, arrowed). Note co-localised PET uptake in (B, arrowed). CT allows assessment of plaque density in (C) (<30 Hounsfield units). Hybrid PET CT in (D) shows co-localisation of 18-fluoride uptake.

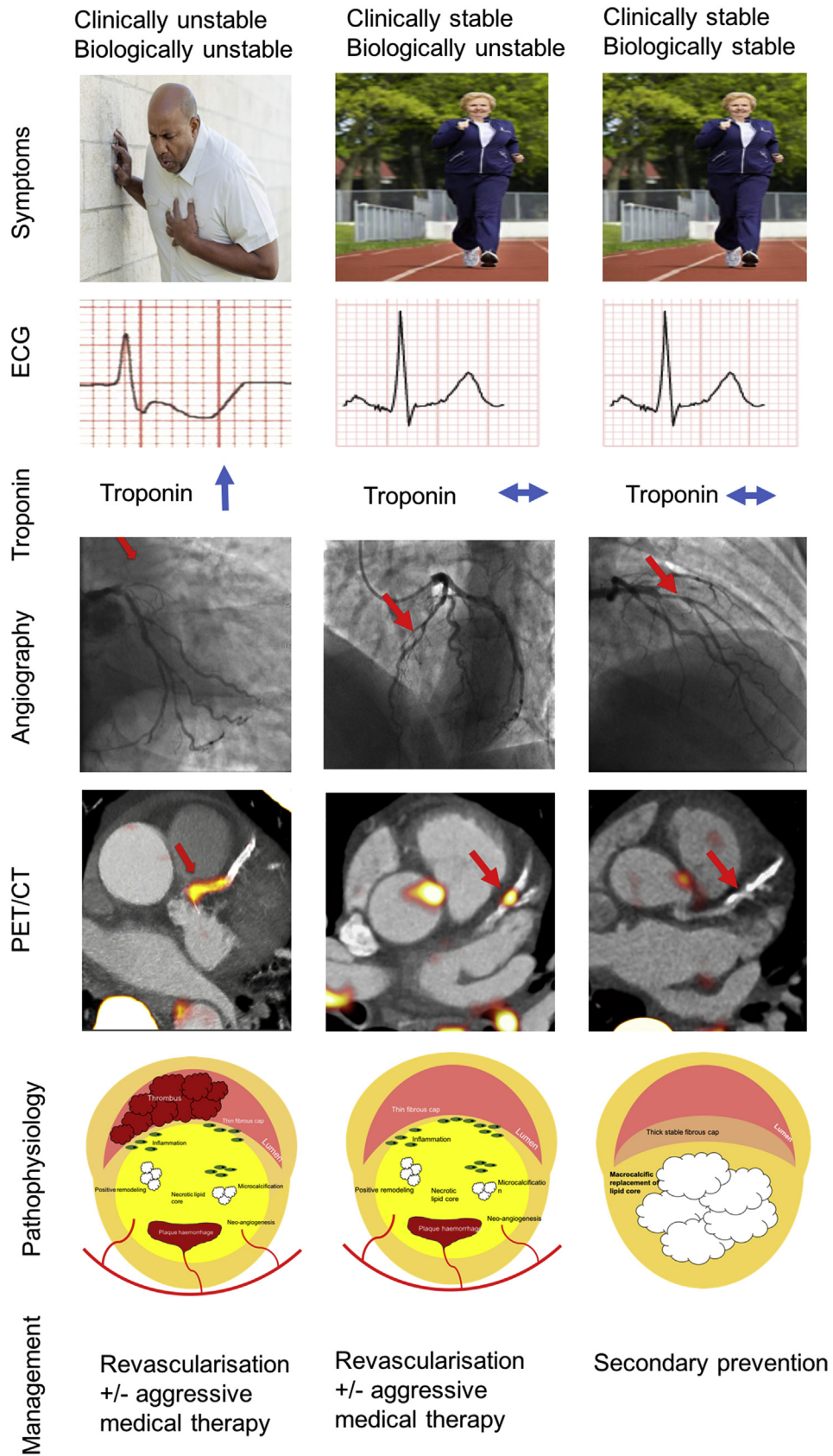


Fig. 6. A potential novel definition of stable and unstable coronary artery disease.

Note: this proposal would require extensive research and investigation before it could be recommended in routine clinical practice. Pending this current guideline, recommendations for revascularisation and the prescription of optimal medical therapy should be followed.

scores for the development of cardiovascular disease were 2.4 times higher amongst adults in the highest quartile of coronary artery 18F-NaF uptake compared with those in the lowest (8.0 vs. 3.3%, $p < 0.001$) [107].

At the plaque level, 18F-fluoride localises to plaques with multiple unstable features, including inflammation, positive remodeling, necrotic core and cell death as assessed by histology, VH-IVUS, OCT and CT imaging [15,108]. Moreover, increased 18F-fluoride uptake has been demonstrated in both culprit coronary and carotid plaques after acute ischemic events. In a study of 40 patients with recent MI, increased uptake localised to the culprit plaque in 93% of patients. Similar findings were observed in a small MR/PET study [109], whilst in the carotid arteries increased 18F-fluoride uptake was observed in the culprit carotid plaque of patients with recent stroke/TIA *versus* controls [110]. This finding was confirmed in a case control study by Vesey et al. ($n = 26$), who also demonstrated increased 18F-fluoride uptake in ipsilateral *versus* contralateral carotid plaques and that once again was associated with unstable plaque features, plaque burden and cardiovascular risk factors [111] (Fig. 5).

Together, this data suggests that 18F-fluoride allows detection of both unstable and culprit plaque with increased coronary uptake being observed in patients with increased disease activity and at increased cardiovascular risk. Whether it provides incremental prospective prediction of cardiovascular events compared to conventional risk scores and CT imaging will be investigated in the prospective, multicenter PREFFIR trial (NCT02278211). Unlike FDG, drug trials evaluating the effects of cardiovascular medicines on 18F-fluoride uptake are lacking, although the on going SALTIRE 2 study is assessing the effects of bisphosphonates and Denosumab on vascular calcification activity in aortic stenosis (NCT 02132026). It should provide proof of concept as to whether the vascular 18F-fluoride signal is modifiable.

1.9. A novel definition of stable and unstable coronary artery disease

Patients with coronary atherosclerosis have traditionally been divided into two groups depending on their symptom pattern. Patients with stable coronary artery disease are classically either asymptomatic or experience predictable exertional angina [112,113]. The assumption has been that, like their symptoms, these patients have stable inactive atherosclerosis at low risk of rupture. By contrast, patients with unstable coronary artery disease describe rapid and unpredictable symptom escalation that reflects a highly active and unstable underlying disease process. Modern imaging technology now allows us to examine coronary plaque characteristics and disease activity directly. These indicate that some apparently stable patients in fact also have an active underlying disease process characterised by the dynamic formation and resolution of multiple unstable coronary plaques. Early data suggest that these clinically stable but biologically active patients may be at increased cardiovascular risk and therefore may benefit from aggressive medical therapy and potentially even revascularisation [107]. Equally, patients with disease that is both clinically and biologically stable could have therapy tailored back potentially avoiding expensive and intrusive revascularisation strategies. Further studies are required to investigate this hypothesis and to investigate whether it is cost-effective before adoption in to clinical practice (Fig. 6).

2. Conclusion

Modern non-invasive imaging using CT, MR and PET now allows identification of unstable characteristics and the direct

measurement of disease activity in the coronary arteries and large vessels. This advance appears set to improve our pathological understanding of atherosclerosis and has the potential to redefine our understanding of what stable and unstable atherosclerosis truly represents. Indeed, ultimately, we may be able to track disease activity and the transition between stable and unstable disease states with time and in response to therapy. Further work is now required to validate these early findings and to investigate whether these novel imaging approaches provide incremental prognostic information.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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