F-18-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques

Citation for published version:

Digital Object Identifier (DOI):
10.1016/S0140-6736(13)61754-7

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
The Lancet

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**Summary**

**Background** The use of non-invasive imaging to identify ruptured or high-risk coronary atherosclerotic plaques would represent a major clinical advance for prevention and treatment of coronary artery disease. We used combined PET and CT to identify ruptured and high-risk atherosclerotic plaques using the radioactive tracers $^{18}$F-sodium fluoride ($^{18}$F-NaF) and $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG).

**Methods** In this prospective clinical trial, patients with myocardial infarction (n=40) and stable angina (n=40) underwent $^{18}$F-NaF and $^{18}$F-FDG PET-CT, and invasive coronary angiography. $^{18}$F-NaF uptake was compared with histology in carotid endarterectomy specimens from patients with symptomatic carotid disease, and with intravascular ultrasound in patients with stable angina. The primary endpoint was the comparison of $^{18}$F-fluoride tissue-to-background ratios of culprit and non-culprit coronary plaques of patients with acute myocardial infarction.

**Findings** In 37 (93%) patients with myocardial infarction, the highest coronary $^{18}$F-NaF uptake was seen in the culprit plaque (median maximum tissue-to-background ratio: culprit 1.66 [IQR 1.40–2.25] vs highest non-culprit 1.24 [1.06–1.38], p<0.0001). By contrast, coronary $^{18}$F-FDG uptake was commonly obscured by myocardial uptake and where discernable, there were no differences between culprit and non-culprit plaques (1.71 [1.40–2.13] vs 1.58 [1.28–2.01], p=0.34). Marked $^{18}$F-NaF uptake occurred at the site of all carotid plaque ruptures and was associated with histological evidence of active calcification, macrophage infiltration, apoptosis, and necrosis. 18 (45%) patients with stable angina had plaques with focal $^{18}$F-NaF uptake (maximum tissue-to-background ratio 1.90 [IQR 1.61–2.17]) that were associated with more high-risk features on intravascular ultrasound than those without uptake: positive remodelling (remodelling index 1.12 [1.09–1.19] vs 1.01 [0.94–1.06]; p=0.0004), microcalcification (73% vs 21%; p=0.002), and necrotic core (25% [21–29] vs 18% [14–22]; p=0.001).

**Interpretation** $^{18}$F-NaF PET-CT is the first non-invasive imaging method to identify and localise ruptured and high-risk coronary plaque. Future studies are needed to establish whether this method can improve the management and treatment of patients with coronary artery disease.

**Funding** Chief Scientist Office Scotland and British Heart Foundation.

**Introduction**

Coronary atherosclerotic plaque rupture is the principal precipitant of acute myocardial infarction and an important cause of sudden cardiac death. Rupture is challenging to predict because most plaques are non-obstructive and are not identified by stress testing or coronary angiography. Atherosclerotic lesions at risk of rupture have certain histopathological characteristics that include positive remodelling, microcalcification, and a large necrotic core. The development of modern molecular imaging techniques targeted at these features could lead to the identification of such high-risk plaques in vivo and guide the development of novel treatment strategies.

Combined PET and CT is a non-invasive imaging technique that brings functional molecular imaging together with precise anatomical information. We have recently reported preliminary PET-CT data using the tracer $^{18}$F-sodium fluoride ($^{18}$F-NaF) as a marker of valvar and vascular calcification activity in patients with aortic stenosis. Other studies have shown the usefulness of $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) as a surrogate of vascular inflammation and macrophage burden. We therefore investigated whether, compared with the current non-invasive gold standard of $^{18}$F-FDG, $^{18}$F-NaF uptake could identify ruptured and high-risk atherosclerotic plaques in patients with symptomatic coronary and carotid artery disease.

**Methods**

**Patients**

Patients were recruited from the Royal Infirmary of Edinburgh between February, 2012, and January, 2013, in three cohorts: 40 patients with acute ST-segment or non-ST-segment elevation myocardial infarction, 40 patients with stable angina pectoris undergoing elective invasive coronary angiography, and 12 patients (nine evaluable).
undergoing carotid endarterectomy for symptomatic carotid artery disease.7

Exclusion criteria were age younger than 50 years, insulin-dependent diabetes mellitus, women of child-bearing age not receiving contraception, severe renal failure (serum creatinine >250 mmol/L), known contrast allergy, and inability to provide informed consent. Only patients older than 50 years were recruited in the study to reduce any long-term risks associated with radiation exposure. Uncontrolled diabetes and high blood glucose concentrations (>11 mmol/L) interfere with the quality of ¹⁸F-FDG PET imaging because of the competition between glucose and ¹⁸F-FDG for cellular entry. The convention is therefore to exclude such patients from vascular ¹⁸F-FDG PET studies.7,8,12,13

All patients underwent a comprehensive baseline clinical assessment including evaluation of their cardiovascular risk factor profile. Plasma troponin I concentrations were measured in patients with stable angina using the ARCHITECT STAT high-sensitivity troponin I assay (Abbott Laboratories, Abbott Park, IL, USA; lower limit of detection 1·2 ng/L; 99th percentile diagnostic threshold 26 ng/L). Studies were done with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and with the written informed consent of each participant.

Procedures

Patients with myocardial infarction and stable angina underwent ¹⁸F-NaF and ¹⁸F-FDG PET-CT, CT coronary angiography, and CT calcium scoring (appendix).7 To minimise myocardial uptake, patients were instructed to adhere to a low-carbohydrate, high-protein, and high-fat diet for at least 24 h before undergoing ¹⁸F-FDG PET-CT.

Electrocardiograph-gated PET images were reconstructed in diastole (50–75% of the R-R interval, Ultra-HD) using the Siemens Ultra-HD algorithm, fused with the CT coronary angiogram, and analysed by experienced observers blinded to the clinical diagnosis (NJ, MD, FC) using an OsiriX workstation (OsiriX version 5.5.1 64-bit; OsiriX Imaging Software, Geneva, Switzerland). Two-dimensional regions of interest were drawn around all major (diameter >2 mm) epicardial vessels on 3 mm axial slices just beyond the discernible adventitial border. The maximum standard uptake value (the decay corrected tissue concentration of the tracer divided by the injected dose per bodyweight) was measured and corrected for blood pool activity in the superior vena cava to provide tissue-to-background ratio (TBRs) measurements. Using this method, we have previously shown excellent reproducibility for ¹⁸F-NaF TBR measurements in the coronary arteries with an intraclass correlation coefficient of 0.97.

We used a previously established 95% lower reference limit to categorise coronary plaques into ¹⁸F-NaF positive lesions (focal uptake with a TBR more than 25% higher than a proximal reference lesion) and negative plaques if these criteria were not achieved. This limit was based on our previous study, where plaques with high ¹⁸F-NaF uptake had maximum TBRs that were 44% (95% CI 26–62) higher than a proximal quiescent reference lesion.7 In patients with acute myocardial infarction, ¹⁸F-NaF uptake in the culprit plaque was compared with the highest value in any of the non-culprit vessels.

Quantification of ¹⁸F-FDG uptake was performed as for ¹⁸F-NaF uptake but restricted to the proximal and mid-portions of the coronary arteries, and to regions where myocardial uptake and spillover could be confidently excluded.7 Again, ¹⁸F-FDG positive plaques were defined using the 25% threshold as described for ¹⁸F-NaF. Effective myocardial suppression of ¹⁸F-FDG was predefined as a standard uptake value of 5·0 or less in the basal ventricular septum (appendix) as per published data.22

In patients with stable angina, PET-CT imaging was prospectively used to direct greyscale and radio-frequency intravascular ultrasound (20 MHz Eagle Eye Platinum Catheters [Volcano Corp, San Diego, CA, USA], motorised pull-back 0·5 mm/s) to the ¹⁸F-NaF positive and negative plaques. The interventional cardiologist acquiring the intravascular ultrasound data was blinded to the PET-CT status of the plaque.

Intravascular ultrasound analysis was done as described previously22,28 using dedicated VIAS software (Volcano Image Analysis Software version 3.0) by operators blinded to the PET data. Regions of interest were drawn around the external elastic membrane and luminal borders, and plaque area and composition (dense calcium, necrotic core, fibro-fatty tissue, and fibrous tissue) calculated.23,28 The presence of microcalcification (spotty calcification in the absence of acoustic shadowing on three or more consecutive frames) and the maximum frame necrotic core (the highest percentage of necrotic core on a single frame) were recorded.29 The remodelling index was defined as the ratio between the external elastic membrane cross-sectional area of the lesion and a proximal reference region in the same vessel.30 Plaques were classified as thin-cap fibroatheroma, thick-cap fibroatheroma, pathological intimal thickening, or fibrocalcific plaque as defined previously.30,31

CT analysis was done on a dedicated cardiovascular workstation (Vital Images, Minnetonka, MN, USA). Vessel-specific and total Agatston calcium scores were calculated as described previously.7 An independent experienced and blinded observer (MW) determined the stenosis severity, plaque composition (calcified, non-calcified, mixed plaque), and presence of high-risk CT features (positive remodelling, microcalcification, necrotic core) according to standard definitions in plaques with and without increased ¹⁸F-NaF activity.31

Intact atherosclerotic plaques were retrieved at the time of carotid endarterectomy and scanned using ex-vivo PET-CT to allow precise anatomical colocalisation of ¹⁸F-NaF activity with pathological evidence of plaque rupture. Plaques were divided into ¹⁸F-NaF positive and negative areas, and histological sections were assessed
using Movat’s pentachrome and immunohistochemistry to investigate calcification activity (tissue non-specific alkaline phosphatase and osteocalcin), macrophage infiltration (CD68), and cell death (apoptosis, cleaved caspase 3; presence of necrotic core; appendix).

**Statistical analysis**
The primary endpoint of the study was the comparison of $^{18}$F-fluoride tissue-to-background ratios of culprit and non-culprit coronary plaques of patients with acute myocardial infarction. The main secondary endpoints were comparative imaging and histological characterisation of $^{18}$F-fluoride positive and negative atherosclerotic plaques in patients with coronary and carotid artery disease. Based on our previous data, we required 36 patients with myocardial infarction to detect a difference of 0.23 in the tissue-to-background ratio between culprit and non-culprit plaques at 90% power and two-sided $p<0.05$. We recruited 40 patients to account for incomplete data and recruited a similar sized (n=40) comparator group of patients with stable angina.

Continuous data were tested for normality with the D’Agostino-Pearson omnibus test. Continuous parametric variables were expressed as mean (SD) and

### Table 1: Baseline characteristics of patients with coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>Myocardial infarction</th>
<th>Stable angina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=40)</td>
<td>STEMI (n=26)</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>62 (8)</td>
<td>63 (9)</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>37 (93%)</td>
<td>24 (92%)</td>
</tr>
<tr>
<td>Body-mass index (kg/m²), mean (SD)</td>
<td>28 (5)</td>
<td>27 (5)</td>
</tr>
<tr>
<td>Antecedent angina (active), n (%)</td>
<td>9 (23%)</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Heart rate (per min), mean (SD)</td>
<td>56 (7)</td>
<td>56 (7)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg), mean (SD)</td>
<td>132 (21)</td>
<td>131 (20)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg), mean (SD)</td>
<td>76 (3)</td>
<td>76 (9)</td>
</tr>
<tr>
<td>Cardiovascular history, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous MI</td>
<td>5 (13%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Previous CVA/TIA</td>
<td>2 (5%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Previous PCI</td>
<td>5 (13%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>2 (5%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking habit (ex or current)</td>
<td>25 (63%)</td>
<td>19 (73%)</td>
</tr>
<tr>
<td>Non-insulin dependent diabetes</td>
<td>8 (20%)</td>
<td>7 (27%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (43%)</td>
<td>11 (42%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>19 (48%)</td>
<td>11 (42%)</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>40 (100%)</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>39 (98%)</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Statin</td>
<td>39 (98%)</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>β blocker</td>
<td>32 (80%)</td>
<td>20 (77%)</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>35 (88%)</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>2 (5%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Other anti-hypertensive</td>
<td>3 (8%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Oral nitrates</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Serum biochemistry, mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7 (1.2)</td>
<td>4.7 (1.3)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.1 (0.3)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.9 (1.1)</td>
<td>2.8 (1.1)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 (0.8)</td>
<td>1.7 (0.7)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>84 (27)</td>
<td>86 (29)</td>
</tr>
<tr>
<td>Coronary artery calcium score (Agatston units), median (IQR)</td>
<td>159 (42-456)</td>
<td>176 (45-474)</td>
</tr>
<tr>
<td>Peak troponin concentration (ng/L), median (IQR)</td>
<td>32 (200)</td>
<td>11200 (3300-50 000)</td>
</tr>
</tbody>
</table>

NSTEMI=non-ST elevation myocardial infarction. MI=myocardial infarction. CVA=cerebrovascular accident. TIA=transient ischaemic attack. PCI=percutaneous coronary intervention. ACEI=angiotensin converting enzyme inhibitor. ARB=angiotensin receptor blocker. CABG=coronary artery bypass graft. HDL=high-density lipoprotein. LDL=low-density lipoprotein. STEMI=ST elevation myocardial infarction. *Heart rate at the time of CT coronary angiography. †Medications at the time of scan.
angiography was 7 (IQR 1–12) days. The total effective scanning within a median of 6 (IQR 3–9) days. The and ¹⁸F-FDG (90 [7] min after 192 [11] MBq) PET-CT

Patients were predominantly middle-aged men and had multiple cardiovascular risk factors (table 1). They underwent both ¹⁸F-NaF (60 [SD 9] min after 123 [SD 5] MBq) and ¹⁸F-FDG (90 [7] min after 192 [11] MBq) PET-CT scanning within a median of 6 (IQR 3–9) days. The total effective radiation dose from study participation was 13·7 (SD 3·0) mSv (conversion factor of 0·014 mSv/mGy.cm): ¹⁸F-NaF (3·8 [SD 0·3] mSv) and ¹⁸F-FDG (4·9 [0·5] mSv) PET-CT, CT coronary angiogram (3·7 [2·1] mSv), and calcium score (1·3 [0·5] mSv).

The culprit vessel was the left anterior descending artery in 17 (42%) patients, the left circumflex artery in seven (18%), and the right coronary artery in 16 (40%). Patients underwent ¹⁸F-NaF scans 6 (IQR 3–10) days after hospitalisation for myocardial infarction (symptoms to ¹⁸F-NaF scan, 8 [3–10] days). ¹⁸F-NaF activity in the culprit plaque was 34% higher than the maximum activity recorded anywhere else in the coronary vasculature (maximum TBR 1·66 [1·40–2·25] vs 1·24 [1·06–1·38], p<0·0001; figures 1 and 2). In 37 of the 40 patients (93%), increased ¹⁸F-NaF uptake was seen in the culprit plaque (figure 1; appendix). In the three patients without uptake, two were younger smokers (aged 50 and 52 years) and, in the third, the culprit lesion was adjudicated as the right coronary artery although focal increased activity was seen in the left circumflex artery. In five patients, increased ¹⁸F-NaF activity was seen at multiple sites within the coronary circulation.

Predefined myocardial suppression of ¹⁸F-FDG uptake was achieved in 28 (70%) patients (median myocardial standard uptake value 3·92 [IQR 2·71–5·55]). However, coronary ¹⁸F-FDG uptake could not be distinguished from patchy myocardial uptake in 22 patients affecting 52% of vessel territories. Increased uptake of ¹⁸F-FDG was observed in the culprit vessels of six (33%) of the remaining 18 patients. Overall, no significant differences could be shown between the maximum TBRs in the culprit plaques and those recorded elsewhere in the coronary vasculature (1·71 [IQR 1·40–2·13] vs 1·58 [1·28–2·01], p=0·34; figure 2) with a mean difference of 0·09 (95% CI –0·07 to 0·24).

The median duration between clinical symptoms and carotid endarterectomy was 17 (IQR 10–27) days (appendix). Carotid endarterectomy specimens (figure 3; appendix) were obtained for 12 patients, although three specimens could not be excised intact and were discarded. Ex-vivo ¹⁸F-NaF PET-CT was undertaken in nine specimens and uptake was localised to the site of macroscopic plaque rupture in all patients (figure 3). Compared with sections of tissue without uptake (n=15), those with increased ¹⁸F-NaF uptake (n=24) had increased calcification activity (tissue non-specific alkaline phosphatase 4·07% [SD 3·42] vs 0·76% [0·51], p=0·001; osteocalcin 1·88% [IQR 0·58–4·10] vs 0·25% [0·11–0·58], p<0·0001), macrophage infiltration (CD68, 350 [IQR 172–840] vs 145 [24–362] cells/mm², p=0·013), and cell death (apoptosis, cleaved-caspase-3, 1·23% [0·69–1·91] vs 0·09% [0·04–1·38], p=0·005; necrotic core, 22/4 vs 4/15; p=0·0001; appendix).

Patients with stable angina were older (67 [SD 8] vs 62 [8] years, p=0·006) and had more severe coronary artery disease (coronary artery calcium score 599 [IQR 60–1302] vs 159 [42–456] Agatston units, p=0·006) than those with myocardial infarction (table 1). Focal ¹⁸F-NaF uptake was noted in 18 patients (45%), which did not seem to be related to percutaneous coronary intervention and stent Would you like to continue with the rest of the content?
deployment (appendix). The maximum TBR for $^{18}$F-NaF positive plaques was $1.90$ [IQR 1.61–2.17] and for $^{18}$F-NaF negative plaques was $1.02$ [0.82–1.17]. $^{18}$F-NaF positive plaques were predominantly (72% of patients) non-obstructive (<70% luminal stenosis) on coronary angiography and showed multiple high-risk features on radiofrequency intravascular ultrasound (positive remodelling [remodelling index 1.12 [IQR 1.09–1.19] vs
positive lesions had higher concentrations of plasma troponin at baseline (3.35 [IQR 2.35–10.20] vs 2.45 [1.85–4.02] ng/L; p=0.047), with one individual having a concentration (35 ng/L) above the 99th percentile diagnostic threshold.

Although predefined myocardial suppression of ¹⁸F-FDG uptake was achieved in 34 (85%) patients (median myocardial standard uptake value 2.60 [IQR 1.83–3.83]), coronary ¹⁸F-FDG uptake could not be confidently interpreted in 45% of vessel territories. Increased focal ¹⁸F-FDG uptake was noted in just four patients: three at the site of recent coronary stenting and one at the ostium of a saphenous vein graft.

**Discussion**

We have shown that intense ¹⁸F-NaF uptake localises to recent plaque rupture in patients with acute myocardial infarction and in those with symptomatic carotid disease. Moreover, in patients with stable coronary artery disease, ¹⁸F-NaF uptake seems to identify coronary plaques with high-risk features on intravascular ultrasound. This technique holds major promise as a means of identifying high-risk and ruptured plaque, and potentially informing the future management and treatment of patients with stable and unstable coronary artery disease.

**Figure 2: ¹⁸F-fluoride and ¹⁸F-fluorodeoxyglucose uptake in patients with myocardial infarction**

¹⁸F-fluoride activity (maximum tissue-to-background ratio) was increased in the culprit plaque (red) compared with the maximum uptake in any of the non-culprit plaques (blue). By contrast, there was no difference in the activity of ¹⁸F-fluorodeoxyglucose between these regions.

**Figure 3: Carotid ¹⁸F-fluoride uptake and carotid plaque rupture**

In-vivo (A and B) and ex-vivo (C and D) positron emission and computed tomograms showing colocalisation of ¹⁸F-fluoride (¹⁸F-NaF) uptake (yellow-orange) to the site of plaque rupture with adherent thrombus on excised carotid endarterectomy tissue (E and F). Histology of the ¹⁸F-NaF-positive region shows a large necrotic core (Movat’s pentachrome, magnification 4×, G), within which increased staining for tissue non-specific alkaline phosphatase can be seen as a marker of calcification activity on immunohistochemistry (magnification 4×, H; magnification 10×, I).
Over 90% of our patients with myocardial infarction had increased $^{18}$F-NaF uptake at the site of their culprit ruptured plaque, with TBR values that were a third higher than the maximum activity anywhere else in the coronary vasculature. These findings were not unique to the coronary circulation since we also noted increased focal $^{18}$F-NaF uptake at the site of plaque rupture in all excised carotid endarterectomy specimens from patients with symptomatic carotid disease. However, we do acknowledge that this was not a universal finding. Of the three patients with myocardial infarction who had no uptake, two were younger smokers with only mild underlying irregularities on coronary angiography, implicating plaque erosion and thrombosis as the mechanism of their infarction rather than plaque rupture. The third patient sustained an inferolateral non-ST segment elevation myocardial infarction and had a lesion stented in the right coronary artery. Increased $^{18}$F-NaF activity was seen in the co-dominant circumflex artery that could have equally explained the clinical presentation, raising the intriguing possibility that $^{18}$F-NaF might have a clinical role for patients in whom the culprit lesion is not readily apparent.

Focal regions of increased $^{18}$F-NaF activity were seen in almost a half of our patients with stable coronary artery disease. To understand the mechanism of uptake in these patients, we sought to compare plaque characteristics of lesions with and without increased $^{18}$F-NaF uptake. Because histology of the coronary arteries in this population is not feasible, we undertook greyscale and radiofrequency intravascular ultrasound, a widely used and validated process that provides detailed characterisation of plaque composition. This method showed that lesions with increased $^{18}$F-NaF uptake were associated with greater positive remodelling, more microcalcification, and a larger necrotic core. These findings were corroborated by, and consistent with, the findings of plaque analysis done with CT coronary angiography. Plasma troponin concentrations measured by a novel high-sensitivity assay were also higher in those patients with $^{18}$F-NaF positive plaques than in patients with $^{18}$F-NaF negative plaques, perhaps implicating subclinical plaque rupture with embolisation and microinfarction.

Why does $^{18}$F-NaF bind to ruptured or high-risk plaque? Similar to the caseating granuloma of tuberculosis, atherosclerotic vascular calcification is a controlled cellular response to an intense, necrotic, and chronic inflammatory stimulus. Indeed, direct links between inflammatory cells and osteoblastic metaplasia in the vasculature are well described. Hydroxyapatite is the central structural component of vascular calcification and is laid down during the earliest and most active stages of mineralisation: hydroxyapatite nanocrystals nucleate, propagate, and mineralise the extracellular matrix. Fluoride ions are incorporated into the hydroxyapatite by ion exchange with hydroxyl groups at the crystal surface. This process is dependent on the crystal surface area that will be greatest in the earliest and most active nanocrystalline stages of mineralisation associated with plaque inflammation and necrosis. We believe that these processes are responsible for the observed $^{18}$F-NaF uptake and is consistent with our data showing $^{18}$F-NaF uptake in regions of necrosis, macrophage infiltration, apoptosis, microcalcification, and alkaline phosphatase and osteocalcin staining. Moreover, mathematical modelling indicates that microcalcification at the surface of thin-capped atheroma (figure 1) can intensify and double incident stresses. Microcalcification is therefore not only a marker of acute plaque rupture but is implicated in its precipitation.

Coronary arterial calcification is considered pathognomonic of atherosclerosis and is a powerful independent risk predictor for cardiovascular events that can be further refined by the rapidity of its progression. Why then not rely on CT coronary calcium scoring alone as a biomarker? Microcalcification cannot be detected on CT and confluent coronary macrocalcification develops slowly, taking many months or years to become apparent on CT, and can become dormant once inflammation in
Systematic review

We searched PubMed using variations of the keywords “high-risk plaques”, “vulnerable plaques”, “ruptured plaques”, “¹⁸F-fluorodeoxyglucose positron emission tomography”, “¹⁸F-fluoride positron emission tomography”, and “coronary arteries”. The search was restricted to human studies. We assessed the quality of the evidence specifically related to cardiovascular disease by reviewing the patient population studied and the methodology for the positron emission and CT imaging.

Non-invasive imaging of carotid plaque inflammation using ¹⁸F-fluorodeoxyglucose positron emission tomography was reported by Rudd and colleagues in 2002. Since then, this tracer has been validated and widely used as a surrogate of large vessel inflammation. Increased ¹⁸F-fluorodeoxyglucose in the coronary arteries has been described in patients with coexisting malignancy. Since then, three prospective studies have examined the feasibility and reproducibility of assessing uptake of this tracer in the coronary vasculature. Only two small studies (n=10–20) have suggested that ¹⁸F-fluorodeoxyglucose might identify some inflamed plaques in patients with recent myocardial infarction, although the largest study showed that in 50% of patients with acute myocardial infarction, there was no uptake of ¹⁸F-fluorodeoxyglucose in the culprit plaque.

Four retrospective studies in patients with cancer have recently reported cardiovascular uptake of ¹⁸F-fluoride. The aortic uptake of ¹⁸F-NaF was first reported by Derlin and colleagues and cardiac ¹⁸F-fluoride uptake by Beheshti and colleagues. We reported the coronary uptake of ¹⁸F-NaF in a prospective clinical trial involving patients with aortic stenosis, and these results were subsequently corroborated by Li and colleagues in their retrospective study of patients with cancer. No study has prospectively assessed this tracer in patients with stable or unstable coronary artery disease or validated its activity against histology or invasive intracoronary imaging, such as intravascular ultrasound. There are no previous reports of ¹⁸F-fluoride uptake in relation to plaque vulnerability or rupture.

Interpretation

There are currently no non-invasive imaging techniques that can identify high-risk and ruptured coronary atherosclerotic plaques in vivo in patients with coronary heart disease. For the first time, we have shown that ¹⁸F-fluoride positron emission tomography can identify culprit and ruptured plaques in patients with myocardial infarction and symptomatic carotid disease. Moreover, histological characterisation demonstrates that ¹⁸F-fluoride activity localises to regions of plaque rupture with evidence of increased inflammation, calcification activity, necrosis, and cell death. In patients with stable angina, ¹⁸F-fluoride is associated with coronary plaques that have high-risk features on intravascular ultrasound, including positive remodelling, microcalcification, and necrosis. Given its ability to identify high-risk or ruptured coronary atherosclerotic plaque, this non-invasive imaging technique has the potential to change how we identify, manage, and treat patients with stable and unstable coronary artery disease. Further work is now needed to establish whether ¹⁸F-fluoride positron emission tomography will provide a means of improving risk stratification, monitoring disease progression, guiding therapeutic interventions, and assessing novel anti-atherosclerotic therapies.

The plaque has subsided. By identifying areas of nascent and ongoing calcification activity, ¹⁸F-NaF uptake allows us to detect regions of metabolically active plaque, thus providing complementary information to CT. Indeed, we noted large areas of coronary CT calcium in the absence of increased ¹⁸F-NaF uptake (figure 1) whereas other regions with minimal or no CT calcium had intense ¹⁸F-NaF uptake (appendix) in keeping with previous observations in the aorta by Derlin and colleagues (panel). Moreover, given that ¹⁸F-NaF seems more closely aligned with the process of necrotic inflammation and plaque metabolic activity, we believe that it potentially offers major improvements to the prediction of cardiovascular risk compared with calcium scoring.

Our data have already established that ¹⁸F-NaF identifies plaque with multiple high-risk features, but prospective studies are now needed in a broad range of patients to assess whether increased coronary ¹⁸F-NaF activity will ultimately translate into future adverse events. If the results prove confirmatory then this technique has the potential to fundamentally alter the way we treat coronary artery disease: moving us away from the current framework based on lesion severity and ischaemia to one focused on plaque metabolism and inflammation. It could, for example, permit the identification of the vulnerable patient with single or multiple high-risk or silently ruptured plaques, providing an opportunity to treat and modify their risk to prevent future adverse cardiovascular events.

By contrast with ¹⁸F-NaF, ¹⁸F-FDG imaging was hampered by problems related to tracer uptake in the myocardium. Our stringent dietary recommendations resulted in suppression of myocardial activity in 70–85% of patients: a rate that compares favourably with previous studies (57–84%). However, this suppression resulted in a patchy distribution of myocardial uptake that frequently obscured activity in one or more coronary vessels. Increased ¹⁸F-FDG uptake might possibly occur in the culprit plaque and we failed to show this because of incomplete data or the delay in scanning. However, given its limitations, we believe that ¹⁸F-FDG is unlikely to become sufficiently robust to permit its clinical application to the coronary circulation. Nevertheless, ¹⁸F-FDG uptake remains an important measure of general vascular inflammation in the aorta and carotid arteries, providing complementary and distinct metabolic information to that of ¹⁸F-NaF uptake.

We acknowledge that there are limitations of our study that include a lack of respiratory gating, partial volume artefacts, and the use of surrogate measures for coronary histology. However, we believe that the totality of our comprehensive evidence using multiple approaches and imaging modalities provides a robust and cogent argument to support our contention that ¹⁸F-fluoride uptake identifies vulnerable and high-risk plaques in patients with stable and unstable coronary heart disease. Further work is now needed to establish whether ¹⁸F-NaF PET-CT will provide a clinically useful technique capable of improving risk stratification, monitoring disease progression, guiding therapeutic interventions, and assessing novel anti-atherosclerotic therapies.

Contributors NVJ designed the study, undertook experiments, analysed results, and interpreted the data. ATV undertook experiments, analysed and interpreted the data, and prepared the report. MWHB, NGU, MVM, ASLM, FHM, SEY, AMF, EJVB, and KAAF collected, analysed, and interpreted data, and prepared the report. DAF, NGU, MWBH, NLMC, and NLM collected the data and prepared the report. JHFR, MRD, and DEN contributed to the study design, supervision, and interpretation of data. DEN is the chief
investigator for the study and obtained funding for all studies. All authors participated in data interpretation. NVJ drafted the first and subsequent versions of this report with key input from MRD and DEN, and revisions from all authors, who reviewed and approved the final submitted report.

Conflicts of interest

NLM has received honoraria for Abbott Diagnostics and acted as a consultant for Abbott Diagnostics. The other authors declare that they have no conflicts of interest.

Acknowledgments

The study was funded by the Chief Scientist Office, Scotland (ETM/160) and the British Heart Foundation (PG/12/8/29371). MRD, NLM, and DEN are supported by the British Heart Foundation (C14/09/002, FS/10/024, FS/10/026). JHFR and PAC are part-funded by the NHRI Cambridge Biomedical Research Centre and the British Heart Foundation. The Wellcome Trust Clinical Research Facility and the Clinical Research Imaging Centre are supported by NHS Research Scotland (NRS) through NHS Lothian. We acknowledge the support of staff at the Edinburgh Heart Centre at the Royal Infirmary of Edinburgh, the radiography and radiochemistry staff of the Clinical Research Imaging Centre, and the histology staff at the Queens Medical Research Institute.

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