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^{18}F-Sodium Fluoride (^{18}F-NaF) for Imaging Microcalcification Activity in the Cardiovascular System

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ABSTRACT: Accumulating preclinical and clinical evidence suggests that calcification is one of the body's primary responses to injury and a key pathological feature of cardiovascular disease. Calcification activity can now be imaged using ^{18}F-sodium fluoride (^{18}F-NaF) positron emission tomography (PET) in combination with either computed tomography or magnetic resonance. These techniques allow visualization of calcification activity and, therefore, provide different information to the established macroscopic calcium imaged with computed tomography. Indeed, ^{18}F-NaF PET has been used to investigate a wide range of valvular conditions, including aortic stenosis, mitral annular calcification, and bioprosthetic valve disease, as well as vascular conditions, including abdominal aortic aneurysm disease, coronary, and carotid atherosclerosis, peripheral vascular disease, and erectile dysfunction. In this brief review, we will focus on how ^{18}F-NaF PET has improved our pathophysiological understanding of cardiovascular calcification and how it can be used as a marker of vascular calcification, providing a useful tool that can be utilized in clinical trials investigating the prediction of both disease progression and clinical events. Finally, we will discuss how ^{18}F-NaF might be employed clinically to improve patient assessment and to guide decision-making.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: ^{18}F-NaF ■ atherosclerosis ■ erectile dysfunction ■ positron emission tomography ■ tomography ■ vascular calcification

Calcification is a key feature of atherosclerosis, heart valve disease, and peripheral vascular disease, that is governed in a highly regulated manner with similarities to skeletal bone formation. Computed tomography (CT), which detects large macroscopic deposits of calcium, has until recently been the only available noninvasive imaging modality allowing visualization of this process.[1] However, imaging of this late stage means that the processes leading to calcium formation have often resolved. There is, therefore, interest in imaging the earlier stages of microcalcification where the disease process is active and therapeutic intervention may be more effective.

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Over the last 10 years, ^{18}F-sodium fluoride (^{18}F-NaF) positron emission tomography (PET) has emerged as a noninvasive quantitative imaging modality capable of imaging calcification activity in the vasculature. This has provided significant insight into the underlying pathophysiology of multiple different cardiovascular disease states and unique information regarding disease activity that may prove clinically useful in the future. In this brief review, we will talk about how ^{18}F-NaF PET works and how this novel approach has been used to investigate a range of valvular and vascular diseases including aortic stenosis, mitral annular calcification

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Nonstandard Abbreviations and Acronyms

CT	computed tomography
PET	positron emission tomography
PREFFIR	Prediction of Recurrent Events With 18F-Fluoride

(MAC), bioprosthetic valve degeneration, abdominal aortic aneurysm disease, erectile dysfunction, carotid atherosclerosis, and coronary artery disease (Figure 1).

Highlights

- ^{18}F-sodium fluoride positron emission tomography can be used to study calcification activity across the vasculature.
- It has been used to study (1) coronary artery disease, (2) carotid atherosclerosis, (3) abdominal aortic aneurysm disease, (4) aortic stenosis, (5) bioprosthetic valve degeneration and (6) erectile dysfunction.
- Ongoing clinical trials are evaluating its ability to predict both disease progression and clinical events and to act as a biomarker evaluating drug efficacy.

UNDERSTANDING HOW ^{18}F-NAF PET WORKS

CT is currently only able to identify macroscopic deposits of calcium with a diameter between 200 and 500 µm (CT calcium scoring).[2,3] However, the ^{18}F-NaF signal is higher in areas of microcalcification (<50 µm) than macrocalcific deposits for a similar mass of calcium. Newly developing calcium, adjacent or remote from macrocalcific deposits will pass through this microcalcification stage so that ^{18}F-NaF effectively provides a marker of calcification activity, detecting new calcium formation beyond the resolution of CT.[1,4] Initially used as bone tracer with increased uptake observed in conditions associated with high bone turnover and new bone formation,[5–7] ^{18}F-NaF demonstrates favorable pharmacokinetic properties and has established an excellent safety profile. Following intravenous injection, roughly 70% of ^{18}F-NaF is plasma-based, with the remaining 30 % found in erythrocytes. Because of its small size and negligible protein binding, one hour after administration only ≈10% of the injected ^{18}F-NaF dose remains in the blood (SUVmean [SD]=1.13 [0.05] in right atrium).[8–10] The mechanism of ^{18}F-NaF uptake in bone is well understood. First, it diffuses via the capillary network into the bone extracellular fluid, and then it exchanges with hydroxyl groups on exposed regions of hydroxyapatite crystals on the bone surface to form fluorapatite.[1] The intensity of the tracer uptake and as a result of the PET signal depends mainly on 2 factors: the bone blood flow and the surface area of exposed hydroxyapatite.[1]

Hydroxyapatite is also a key feature of vascular calcification, with a growing literature demonstrating that similar mechanisms govern ^{18}F-NaF PET binding in the cardiovascular system. However, here blood flow is likely to be fairly constant, so the surface area of hydroxyapatite appears to be the major factor affecting ^{18}F-NaF uptake. Indeed ^{18}F-NaF binding is highest in areas of microcalcification compared with large macroscopic deposits due to the very high surface area of hydroxyapatite in these regions of powdery microcalcification.[1,5,6,11] Unlike other PET tracers, ^{18}F-NaF also demonstrates very low uptake in the myocardium, well below that observed even in the blood pool. This important characteristic facilitates clear visualization of regions of increased ^{18}F-NaF uptake in areas of active calcification in the heart but also mediates precise coregistration via alignment of the ventricular blood pools on PET and contrast CT.[10,12–14]

Validation of the cardiovascular ^{18}F-NaF signal has now been provided in multiple independent studies. Using excised atherosclerotic plaque, Cocker et al[15] demonstrated the association between cardiovascular ^{18}F-NaF PET uptake and the histological staining of hydroxyapatite with Goldner trichome. Aikawa et al[16] confirmed that ^{18}F-NaF binds predominantly to hydroxyapatite, whereas in the aortic valve, ^{18}F-NaF has demonstrated a close association with areas of tissue with positive histochemical staining for alkaline phosphatase (r=0.65; P=0.04) and areas with positive immunohistochemical reactivity for osteocalcin (r=0.68; P=0.03).[1,17] Irkle et al[18] demonstrated that ^{18}F-NaF binding is increased in regions of microcalcification, rather than large macroscopic deposits. This is predominantly due to surface area effects and because much of the hydroxyapatite crystal is internalized in the center of macroscopic deposit and, therefore, not available for ^{18}F-NaF binding. Creager et al,[1] confirmed that ^{18}F-NaF binds to areas of microcalcification beyond the resolution of x-ray CT and that this binding was proportional to the surface area of hydroxyapatite in a controlled ex vivo model. Recently, Youn et al[19] confirmed ^{18}F-NaF binding to microcalcifications in the coronary arteries as well as the carotids.

In effect, ^{18}F-NaF-PET detects the surface area of hydroxyapatite crystal and, therefore, provides different information to x-ray CT, which detects tissue density. Put another way, ^{18}F-NaF detects microcalcifications and areas of calcification activity, whereas CT detects establish macroscopic deposits of calcium. The difference information provided by ^{18}F-NaF PET compared with CT has now been demonstrated in multiple human studies in vivo across a range of different conditions, as discussed below. However, this observation was perhaps first described by Derlin et al[20] in a study of 75 patients undergoing whole-body ^{18}F-NaF PET/CT. They demonstrated that in the aorta, carotids, and femoral arteries, only 12% of all calcified plaques on CT demonstrated increased ^{18}F-NaF uptake, whereas 75% of patients demonstrated increased ^{18}F-NaF uptake remote from calcified CT plaques.

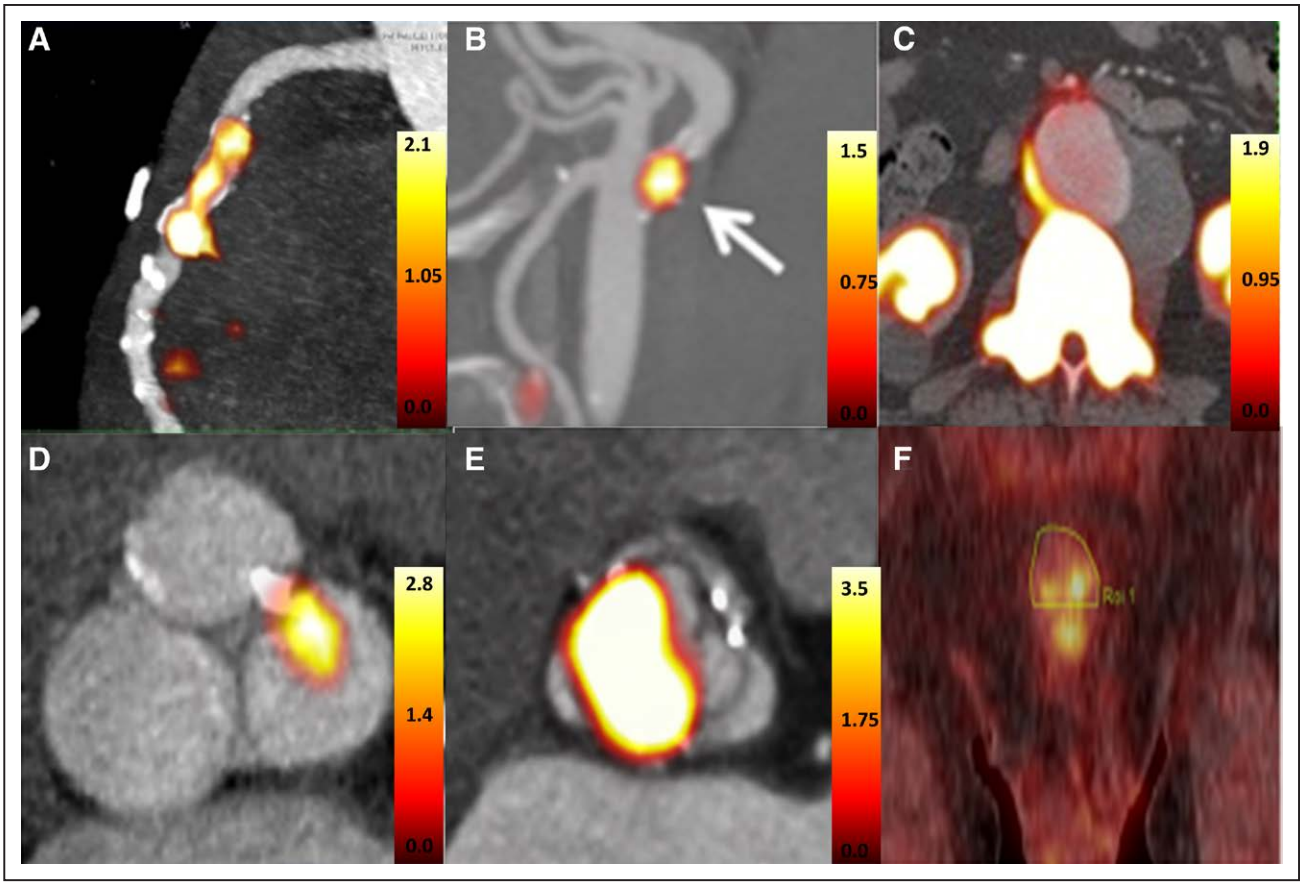

Figure 1. ^{18}F-fluoride uptake in different disease states.
A, Patient with recent myocardial infarction (reprinted from Joshi et al.[34] Copyright ©2014, the Authors), (**B**) patient with recent transient ischemic attack and uptake in the culprit carotid artery (reprinted from Vesey et al.[39] Copyright ©2014, the Authors), (**C**) patient with abdominal aortic aneurysm (reprinted from Forsythe et al.[42] Copyright ©2018, the Authors), (**D**) patient with mild aortic valve stenosis and corresponding ^{18}F-NaF uptake in areas of leaflet calcification (image derived from Dweck et al[17]), (**E**) patient with aortic valve degeneration and corresponding ^{18}F-NaF uptake (reprinted from Cartlidge et al.[29] Copyright ©2019, the Authors), and (**F**) patient with erectile dysfunction and high penile uptake (reprinted from Nakahara et al.[44] Copyright ©2019, Elsevier).

VALVULAR APPLICATIONS

Aortic Stenosis and Bioprosthetic Valve Degeneration

Calcific aortic stenosis is the most common form of valve disease in the Western world and is set to become an increasing health burden with no available effective medical therapy.[21] The only available treatment for patients that progress to severe symptomatic aortic stenosis remains surgical or transcatheter aortic valve replacement. There is, therefore, a pressing need to improve our pathophysiological understanding of the disease and to develop effective medical therapies capable of slowing disease progression.[22,23]

Dweck et al[24] recruited 121 patients with a range of calcific aortic valve disease who underwent both ^{18}F-FDG (fluorodeoxyglucose; a marker of inflammation) and ^{18}F-NaF PET/CT imaging. ^{18}F-NaF uptake activity was higher in aortic stenosis patients than controls and increased progressively with more advanced stages of aortic stenosis. Interestingly increased ^{18}F-FDG activity was also detected, but uptake values were lower than ^{18}F-NaF and had only a mild association with disease severity. Particularly, in the more advanced stages of the disease, calcification, rather than inflammation, had a more predominant role in the disease progressionr[25] On this basis, we think it should be the target of future therapeutic interventions, although acknowledge that other processes such as fibrosis also determine valve stiffening and progression. Once again, in aortic stenosis, ^{18}F-NaF activity was observed in a different distribution to the presence of calcium on CT. Moreover, ^{18}F-NaF PET predicted where new areas of calcium on CT would develop on repeat scans performed 2 years later (Figure 2), consistent with it acting as marker of calcification activity and, therefore, providing powerful prediction of disease progression and future aortic valve replacement or cardiovascular mortality.[26,27]

Calcification also appears to have an important role in bioprosthetic valve degeneration, acting as a major pathological contributor to both progressive bioprosthetic valve narrowing and leaflet tears.[28] Cartlidge et al[29] examined 15 failed explanted bioprosthetic aortic valves using ^{18}F-NaF

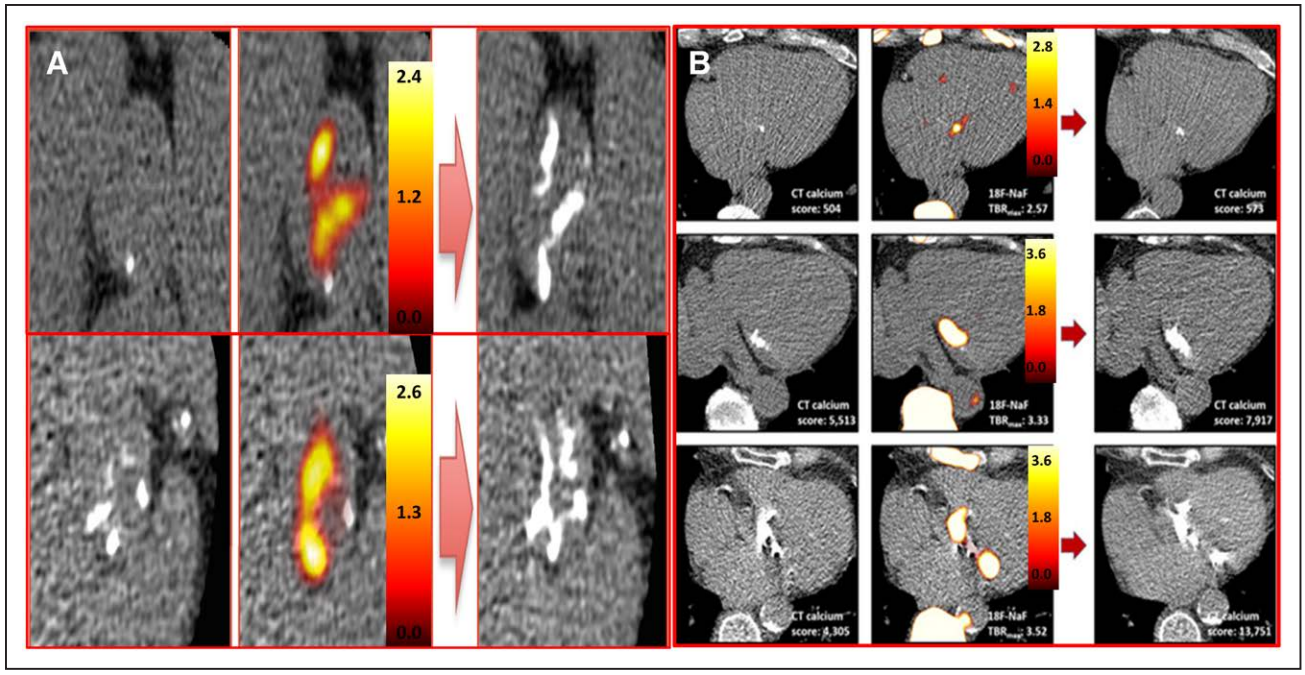

Figure 2. ^{18}F-sodium fluoride (^{18}F-NaF) uptake predicts progression of aortic valve calcification and mitral annular calcification (MAC).

A, Baseline calcium score (**left**) of 2 patients with aortic sclerosis (**top**) and moderate aortic stenosis (**bottom**). Fused ^{18}F-NaF positron emission tomography (PET)–computed tomography (CT) scans (**middle**) show fluoride uptake in red and yellow. Follow-up CT at 2 y (**right**) indicated new areas of macroscopic calcium on the repeat CT in a similar distribution to the PET activity (reprinted from Jenkins et al[47] with permission. Copyright ©2015, the Journal of the American College of Cardiology). **B**, Baseline calcium score (**left**) of patients with mild, moderate, and severe MAC. Fused ^{18}F-NAF PET-CT scans (**middle**) show fluoride uptake in red and yellow. Similar to the aortic valve the baseline PET predicts where new macroscopic calcium in the mitral valve will develop on follow-up CT at 2 y (**right**) (reprinted from Massera et al.[44] Copyright ©2019, the Authors).

PET/CT. All valves demonstrated ^{18}F-NaF leaflet uptake that correlated with microcalcific and macrocalcific deposits within the valve leaflets and colocalized with regions of tissue degradation, pannus, and thrombus on histology. Moreover, they examined 71 patients without known bioprosthetic valve dysfunction. Similar to the ex vivo findings, increased ^{18}F-NaF uptake colocalized with areas of spotty calcification, noncalcific leaflet thickening (suggestive of thrombus), and pannus observed on the CT. Twenty-four patients with increased ^{18}F-NaF uptake at baseline demonstrated clear evidence of deteriorating bioprosthesis function after 2 years, whereas patients without uptake displayed no change in valve function. Ten patients developed new bioprosthetic valve failure; all 10 patients had increased ^{18}F-NaF uptake at baseline.

Mitral Annular Calcification

MAC is a chronic process, the incidence of which is associated with advanced age, atherosclerosis, and altered mineral metabolism. It can lead to mitral valve dysfunction and is associated with an increased risk of adverse cardiovascular events; however, its pathophysiology remains incompletely understood.[30,31] Massera et al[32] investigated MAC using ^{18}F-NaF and ^{18}F-FDG positron emission tomography. The results were similar to those observed in aortic stenosis. Uptake of both tracers was higher in patients with MAC versus those with normal mitral valves and increased with disease severity. A strong correlation was observed between the baseline CT-MAC score and mitral annular ^{18}F-NaF activity (r=0.79, P<0.001), whereas a moderate correlation was observed with ^{18}F-FDG uptake (r=0.32, P=0.001). Similar to the aortic valve, areas of increased ^{18}F-NaF activity on baseline imaging predicted where new regions of macroscopic calcium on CT would develop after 2 years. Indeed, there was a close association observed between baseline ^{18}F-NaF and the change in CT calcium score (r=0.75, P<0.001), with ^{18}F-NaF again emerging as a marker of disease activity and predictor of disease progression in this condition.

VASCULAR APPLICATIONS

Coronary and Carotid Atherosclerosis

Dweck et al[33] first described ^{18}F-NaF uptake in the coronary arteries as a novel marker of microcalcification activity in subjects with and without aortic valve disease. Again ^{18}F-NaF provided different information to CT. More than 40 % of patients with high coronary artery calcium scores (>1000 Agatston Units) did not demonstrate increased ^{18}F-NaF uptake, signifying that ^{18}F-NaF might be able to

differentiate between active disease and advanced yet burnt out atheroma. Increased ^{18}F-NaF uptake could be localized to individual coronary plaques and notably identified patients with both increased cardiac risk scores and a history of prior cardiovascular events. Joshi et al[34] examined 40 patients with stable angina and 40 patients presenting with type 1 myocardial infarction, who all underwent ^{18}F-NaF PET/CT. In the stable cohort, high tracer activity localized to individual coronary plaques in ≈40 % of patients. Using intravascular ultrasound and CT, these plaques had multiple adverse, unstable characteristics, including microcalcification, positive remodeling, and a large necrotic core. In a separate cohort of patients with carotid artery disease undergoing endarterectomy, increased ^{18}F-NaF activity similarly colocalized to carotid plaques with histological adverse plaque features, including increased macrophage accumulation, cell death, and calcification. Finally, in the myocardial infarction cohort, 37 out of 40 patients demonstrated increased ^{18}F-NaF uptake at the exact site of the culprit coronary plaque responsible for the event. By comparison, ^{18}F-FDG was not able to provide similar discrimination of culprit coronary plaques.

Other studies have confirmed the localization of increased ^{18}F-NaF to plaques with adverse characteristics including positive remodeling, low attenuation plaque (<30 HU), spotty calcification, obstructive coronary stenosis, and plaque volumes >100 mm^3 determined by a range of different approaches, including CT, intravascular ultrasound, optical coherent tomography, and histology.[19,35–37] More recently ^{18}F-NaF PET activity has been associated with increased pericoronary adipose tissue density: an emerging marking of coronary plaque inflammation.[38]

Outside the heart, Vesey et al[39] examined ^{18}F-NaF and ^{18}F-FDG uptake in the carotid arteries after transient ischemic attack or stroke. They recruited 26 patients following a recent cerebrovascular event. All individuals underwent PET/CT scanning using both tracers. ^{18}F-NaF uptake was focal, readily identifiable, and localizable to plaque. The authors detected increased ^{18}F-NaF within culprit lesions responsible for stroke compared with both the contralateral nonculprit carotid artery as well as arteries from patients with nonsymptomatic carotid disease. Similar discrimination was again not provided by ^{18}F-FDG. In a smaller study of 9 patients, Quirce et al[40] explored ^{18}F-NaF and ^{18}F-FDG uptake in patients with symptomatic carotid stenosis and showed again that ^{18}F-NaF uptake appeared to be higher in the symptomatic carotid, whereas ^{18}-FDG uptake was nondiscriminatory. Cocker et al[41] examined 11 patients scheduled to have endarterectomy. They performed ^{18}F-NaF PET/ CT imaging of the carotid arteries <2 weeks before surgery and established greater ^{18}F-NaF uptake in symptomatic versus nonsymptomatic plaque.

The retrospective identification of culprit plaque is only of limited clinical utility. More interesting is whether ^{18}F-NaF can prospectively identify patients at increased risk of myocardial infarction so that preventative therapies might be institute to avoid such an event. The prognostic role of ^{18}F-NaF is currently being evaluated in an ongoing prospective multicenter trial: PREFFIR (Prediction of Recurrent Events With ^{18}F-Fluoride; https://www.clinicaltrials.gov; Unique identifier: NCT02278211).

Abdominal Aortic Aneurysms, Peripheral Artery Disease, and Erectile Dysfunction

^{18}F-NaF PET has also been also explored in patients with abdominal aortic aneurysm disease, peripheral vascular disease, and erectile dysfunction. In the SOFIA[3] study (^{18}F-Sodium Fluoride Uptake in Abdominal Aortic Aneurysms), Forsythe et al[42] first performed micro–PET-CT and histological analysis of excised abdominal aortic aneurysm disease tissue, establishing once more that ^{18}F-NaF acts as a marker of microcalcification activity. Then they investigated ^{18}F-NaF PET-CT imaging in patients with abdominal aortic aneurysm disease and control subjects. Increased ^{18}F-NaF uptake was observed in the aneurysms compared with areas of nonaneurysmal aorta, providing important predictive information. Aneurysms in the highest ^{18}F-NaF uptake tertile expanded 2.5× more rapidly than those in the lowest tertile. They were also nearly 3× as likely to experience abdominal aortic aneurysm disease repair or rupture. On multivariable analysis, ^{18}F-NaF provided incremental prognostic information over conventional predictors of risk, including aneurysm size. Interestingly, CT calcium scoring was not predictive of either aneurysm expansion nor clinical events.

In peripheral vascular disease, Chowdhury et al[43] performed a prospective observational study of 50 patients with symptomatic peripheral arterial disease undergoing ^{18}F-FDG and ^{18}F-NaF PET/CT of the superficial femoral artery before and 6 weeks after angioplasty. The investigators found that both tracers performed well in identifying patients who would develop restenosis within 12 months; indeed, there was clear separation of ^{18}F-NaF activity in patients that did and did not go on to develop restenosis.

Finally, Nakahara et al[44] investigated ^{18}F-NaF uptake in the penile arteries of prostate cancer patients undergoing clinical PET scans. The investigators demonstrated that ^{18}F-NaF uptake in the cavernous and dorsal penile arteries was associated with the both the presence of existing erectile dysfunction and the probability of its future development. Indeed, penile ^{18}F-NaF activity was 30% higher in patients with compared with patients without erectile dysfunction.

Limitations

PET-CT has grown significantly over the last decade but still faces important challenges. Most notably is the expense of PET imaging, radiation exposure, and the relatively limited availability of this imaging modality. These

may limit its widespread clinical adoption, although we note the increasing use of PET in oncology. In addition, there are technical challenges. In particular, PET imaging of the heart is complicated by cardiac, respiratory, and gross patient motion and how this effects image quality, particularly in small structures, such as the coronary arteries. Several motion correction methods have been proposed, leading to novel image reconstruction techniques, and the development of new software that can precisely read fused images. The first approach was to ECG-gate the images and only use PET data acquired in diastole when the heart is still (between 50% and 75% of the R-R interval).[14] Although this improves motion artifact, this approach effectively ignores three-fourth of the total PET signal leading to increased noise. Doris et al[45] used an alternative technique that modeled and then corrected for cardiac motion in the PET data using anatomy guided registration algorithm to preserve all of the PET data. Finally, Lassen et al,[46] used an approach that corrected for cardiac, respiratory, and gross patient motion that in combination with background blood pool corrections markedly improved test-retest reproducibility of coronary ^{18}F-NaF PET.

Clinical Trials

Although ^{18}F-NaF PET has provided important pathophysiological insights and emerged consistently as a marker of vascular injury and predictor of disease progression, further work is required to demonstrate the incremental clinical utility of this imaging technique and to justify its relatively high costs. This question is being addressed in multiple prospective studies. As discussed, the PRE^{18}FFIR Study is a multicenter observational study that will follow ≈700 high-risk patients with coronary artery disease to determine whether baseline ^{18}F-NaF PET imaging can identify patients at increased risk of future myocardial infarction and disease progression. SALTIRE 2 (Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression; https://www.clinicaltrials.gov; Unique identifier: NCT02132036) will utilize ^{18}F-NaF PET to determine if denosumab or bisphosphonate can reduce calcification activity and slow aortic stenosis progression in patients with mild and moderate aortic stenosis.

CONCLUSIONS

^{18}F-NaF PET provides a marker of calcification activity across a range of cardiovascular conditions, providing potentially useful prediction of disease progression and clinical events. Although the technique has already provided some key pathophysiological insights and is being used as a surrogate efficacy end point in ongoing randomized clinical trials, large prospective studies are required to examine whether it can provide incremental information to justify its more widespread clinical use.

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Disclosures

None.

REFERENCES

1. Creager MD, Hohl T, Hutcheson JD, Moss AJ, Schlotter F, Blaser MC, Park MA, Lee LH, Singh SA, Alcaide-Corral CJ, et al. 18F-fluoride signal amplification identifies microcalcifications associated with atherosclerotic plaque instability in positron emission tomography/computed tomography images. *Circ Cardiovasc Imaging*. 2019;12:e007835. doi: 10.1161/CIRCIMAGING.118.007835
2. Ritman EL. Small-animal CT - its difference from, and impact on, clinical CT. *Nucl Instrum Methods Phys Res A*. 2007;580:968–970. doi: 10.1016/j.nima.2007.06.040
3. Baheza RA, Welch EB, Gochberg DF, Sanders M, Harvey S, Gore JC, Yankeelov TE. Detection of microcalcifications by characteristic magnetic susceptibility effects using MR phase image cross-correlation analysis. *Med Phys*. 2015;42:1436–1452. doi: 10.1118/1.4908009
4. Bockisch A, Beyer T, Antoch G, Freudenberg LS, Kühl H, Debatin JF, Müller SP. Positron emission tomography/computed tomography–imaging protocols, artifacts, and pitfalls. *Mol Imaging Biol*. 2004;6:188–199. doi: 10.1016/j.mibio.2004.04.006
5. Blau M, Ganatra R, Bender MA. 18 F-fluoride for bone imaging. *Semin Nucl Med*. 1972;2:31–37. doi: 10.1016/s0001-2998(72)80005-9
6. Hawkins RA, Choi Y, Huang SC, Hoh CK, Dahlbom M, Schiepers C, Satyamurthy N, Barrio JR, Phelps ME. Evaluation of the skeletal kinetics of fluorine-18-fluoride ion with PET. *J Nucl Med*. 1992;33:633–642.
7. Czernin J, Satyamurthy N, Schiepers C. Molecular mechanisms of bone 18F-NaF deposition. *J Nucl Med*. 2010;51:1826–1829. doi: 10.2967/jnumed.110.077933
8. Hoh CK, Hawkins RA, Dahlbom M, Glaspy JA, Seeger LL, Choi Y, Schiepers CW, Huang SC, Satyamurthy N, Barrio JR. Whole body skeletal imaging with [18F]fluoride ion and PET. *J Comput Assist Tomogr*. 1993;17:34–41. doi: 10.1097/00004728-199301000-00005
9. Blake GM, Park-Holohan SJ, Cook GJ, Fogelman I. Quantitative studies of bone with the use of 18F-fluoride and 99mTc-methylene diphosphonate. *Semin Nucl Med*. 2001;31:28–49. doi: 10.1053/snuc.2001.18742
10. Moss AJ, Doris MK, Andrews JPM, Bing R, Daghem M, van Beek EJR, Forsyth L, Shah ASV, Williams MC, Sellers S, et al. Molecular coronary plaque imaging using 18F-fluoride. *Circ Cardiovasc Imaging*. 2019;12:e008574. doi: 10.1161/CIRCIMAGING.118.008574
11. Blau M, Nagler W, Bender MA. Fluorine-18: a new isotope for bone scanning. *J Nucl Med*. 1962;3:332–334.
12. Andrews JPM, MacNaught G, Moss AJ, Doris MK, Pawade T, Adamson PD, van Beek EJR, Lucatelli C, Lassen ML, Robson PM, et al. Cardiovascular (18)F-fluoride positron emission tomography-magnetic resonance imaging: a comparison study [published online December 2, 2019]. *J Nucl Cardiol*. https://doi.org/10.1007/s12350-019-01962-y.
13. Kwiecinski J, Berman DS, Lee SE, Dey D, Cadet S, Lassen ML, Germano G, Jansen MA, Dweck MR, Newby DE, et al. Three-hour delayed imaging improves assessment of coronary 18F-sodium fluoride PET. *J Nucl Med*. 2019;60:530–535. doi: 10.2967/jnumed.118.217885

14. Pawade TA, Cartlidge TR, Jenkins WS, Adamson PD, Robson P, Lucatelli C, Van Beek EJ, Prendergast B, Denison AR, Forsyth L, et al. Optimization and reproducibility of aortic valve 18F-fluoride positron emission tomography in patients with aortic stenosis. *Circ Cardiovasc Imaging*. 2016;9:e005131.
15. Cocker MS, Spence JD, Hammond R, deKemp RA, Lum C, Wells G, Bernick J, Hill A, Nagpal S, Stotts G, et al; Canadian Atherosclerosis Imaging Network (CAIN) - Project II. [18F]-Fluorodeoxyglucose PET/CT imaging as a marker of carotid plaque inflammation: comparison to immunohistology and relationship to acuity of events. *Int J Cardiol*. 2018;271:378–386. doi: 10.1016/j.ijcard.2018.05.057
16. Aikawa E, Nahrendorf M, Sosnovik D, Lok VM, Jaffer FA, Aikawa M, Weissleder R. Multimodality molecular imaging identifies proteolytic and osteogenic activities in early aortic valve disease. *Circulation*. 2007;115:377–386. doi: 10.1161/CIRCULATIONAHA.106.654913
17. Dweck MR, Jenkins WS, Vesey AT, Pringle MA, Chin CW, Malley TS, Cowie WJ, Tsampasian V, Richardson H, Fletcher A, et al. 18F-sodium fluoride uptake is a marker of active calcification and disease progression in patients with aortic stenosis. *Circ Cardiovasc Imaging*. 2014;7:371–378. doi: 10.1161/CIRCIMAGING.113.001508
18. Irkle A, Vesey AT, Lewis DY, Skepper JN, Bird JL, Dweck MR, Joshi FR, Gallagher FA, Warburton EA, Bennett MR, et al. Identifying active vascular microcalcification by (18)F-sodium fluoride positron emission tomography. *Nat Commun*. 2015;6:7495. doi: 10.1038/ncomms8495
19. Youn T, Al'Aref SJ, Narula N, Salvatore S, Pisapia D, Dweck MR, Narula J, Lin FY, Lu Y, Kumar A, et al. 18F-sodium fluoride positron emission tomography/computed tomography in ex vivo human coronary arteries with histological correlation. *Arterioscler Thromb Vasc Biol*. 2020;40:404–411. doi: 10.1161/ATVBAHA.119.312737
20. Derlin T, Richter U, Bannas P, Begemann P, Buchert R, Mester J, Klutmann S. Feasibility of 18F-sodium fluoride PET/CT for imaging of atherosclerotic plaque. *J Nucl Med*. 2010;51:862–865. doi: 10.2967/jnumed.110.076471
21. Thaden JJ, Nkomo VT, Enriquez-Sarano M. The global burden of aortic stenosis. *Prog Cardiovasc Dis*. 2014;56:565–571. doi: 10.1016/j.pcad.2014.02.006
22. Brown JM, O'Brien SM, Wu C, Sikora JA, Griffith BP, Gammie JS. Isolated aortic valve replacement in North America comprising 108,687 patients in 10 years: changes in risks, valve types, and outcomes in the Society of Thoracic Surgeons National Database. *J Thorac Cardiovasc Surg*. 2009;137:82–90. doi: 10.1016/j.jtcvs.2008.08.015
23. Brennan JM, Thomas L, Cohen DJ, Shahian D, Wang A, Mack MJ, Holmes DR, Edwards FH, Frankel NZ, Baron SJ, et al. Transcatheter versus surgical aortic valve replacement: propensity-matched comparison. *J Am Coll Cardiol*. 2017;70:439–450. doi: 10.1016/j.jacc.2017.05.060
24. Dweck MR, Jones C, Joshi NV, Fletcher AM, Richardson H, White A, Marsden M, Pessotto R, Clark JC, Wallace WA, et al. Assessment of valvular calcification and inflammation by positron emission tomography in patients with aortic stenosis. *Circulation*. 2012;125:76–86. doi: 10.1161/CIRCULATIONAHA.111.051052
25. Dweck MR, Khaw HJ, Sng GK, Luo EL, Baird A, Williams MC, Makiello P, Mirsadraee S, Joshi NV, van Beek EJ, et al. Aortic stenosis, atherosclerosis, and skeletal bone: is there a common link with calcification and inflammation? *Eur Heart J*. 2013;34:1567–1574. doi: 10.1093/eurheartj/eht034
26. Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Iung B, Lancellotti P, Lansac E, Rodriguez Muñoz D, et al; ESC Scientific Document Group. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J*. 2017;38:2739–2791. doi: 10.1093/eurheartj/ehx391
27. Clavel MA, Burwash IG, Pibarot P. Cardiac imaging for assessing low-gradient severe aortic stenosis. *JACC Cardiovasc Imaging*. 2017;10:185–202. doi: 10.1016/j.jcmg.2017.01.002
28. Cartlidge TR, Pawade TA, Dweck MR. Aortic stenosis and CT calcium scoring: is it for everyone? *Heart*. 2017;103:8–9. doi: 10.1136/heartjnl-2016-310297
29. Cartlidge TRG, Doris MK, Sellers SL, Pawade TA, White AC, Pessotto R, Kwiecinski J, Fletcher A, Alcaide C, Lucatelli C, et al. Detection and prediction of bioprosthetic aortic valve degeneration. *J Am Coll Cardiol*. 2019;73:1107–1119. doi: 10.1016/j.jacc.2018.12.056
30. Barasch E, Gottdiener JS, Larsen EK, Chaves PH, Newman AB, Manolio TA. Clinical significance of calcification of the fibrous skeleton of the heart and aortosclerosis in community dwelling elderly. The Cardiovascular Health Study (CHS). *Am Heart J*. 2006;151:39–47. doi: 10.1016/j.ahj.2005.03.052
31. Fox CS, Vasan RS, Parise H, Levy D, O'Donnell CJ, D'Agostino RB, Benjamin EJ; Framingham Heart Study. Mitral annular calcification predicts cardiovascular morbidity and mortality: the Framingham Heart Study. *Circulation*. 2003;107:1492–1496. doi: 10.1161/01.cir.0000058168.26163.bc
32. Massera D, Trivieri MG, Andrews JPM, Sartori S, Abgral R, Chapman AR, Jenkins WSA, Vesey AT, Doris MK, Pawade TA, et al. Disease activity in mitral annular calcification. *Circ Cardiovasc Imaging*. 2019;12:e008513. doi: 10.1161/CIRCIMAGING.118.008513
33. Dweck MR, Chow MW, Joshi NV, Williams MC, Jones C, Fletcher AM, Richardson H, White A, McKillop G, van Beek EJ, et al. Coronary arterial 18F-sodium fluoride uptake: a novel marker of plaque biology. *J Am Coll Cardiol*. 2012;59:1539–1548. doi: 10.1016/j.jacc.2011.12.037
34. Joshi NV, Vesey AT, Williams MC, Shah AS, Calvert PA, Craighead FH, Yeoh SE, Wallace W, Salter D, Fletcher AM, et al. 18F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial. *Lancet*. 2014;383:705–713. doi: 10.1016/S0140-6736(13)61754-7
35. Kwiecinski J, Dey D, Cadet S, Lee SE, Tamarappoo B, Otaki Y, Huynh PT, Friedman JD, Dweck MR, Newby DE, et al. Predictors of 18F-sodium fluoride uptake in patients with stable coronary artery disease and adverse plaque features on computed tomography angiography. *Eur Heart J Cardiovasc Imaging*. 2020;21:58–66.
36. Kitagawa T, Yamamoto H, Nakamoto Y, Sasaki K, Toshimitsu S, Tatsugami F, Awai K, Hirokawa Y, Kihara Y. Predictive value of 18F-sodium fluoride positron emission tomography in detecting high-risk coronary artery disease in combination with computed tomography. *J Am Heart Assoc*. 2018;7:e010224. doi: 10.1161/JAHA.118.010224
37. Lee JM, Bang JI, Koo BK, Hwang D, Park J, Zhang J, Yaliang T, Suh M, Paeng JC, Shiono Y, et al. Clinical relevance of (18)F-sodium fluoride positron-emission tomography in noninvasive identification of high-risk plaque in patients with coronary artery disease. *Circ Cardiovasc Imaging*. 2017;10:e006704.
38. Kwiecinski J, Dey D, Cadet S, Lee SE, Otaki Y, Huynh PT, Doris MK, Eisenberg E, Yun M, Jansen MA, et al. Peri-coronary adipose tissue density is associated with (18)F-sodium fluoride coronary uptake in stable patients with high-risk plaques. *JACC Cardiovasc Imaging*. 2019;11:30076–30072
39. Vesey AT, Jenkins WS, Irkle A, Moss A, Sng G, Forsythe RO, Clark T, Roberts G, Fletcher A, Lucatelli C, et al. Newby DE. (18)F-fluoride and (18)F-fluorodeoxyglucose positron emission tomography after transient ischemic attack or minor ischemic stroke: case-control study. *Circ Cardiovasc Imaging*. 2017;10:e004976.
40. Quirce R, Martínez-Rodríguez I, Banzo I, Jiménez-Bonilla J, Martínez-Amador N, Ibáñez-Bravo S, López-Defilló J, Jiménez-Alonso M, Revilla MA, Carril JM. New insight of functional molecular imaging into the atheroma biology: 18F-NaF and 18F-FDG in symptomatic and asymptomatic carotid plaques after recent CVA. Preliminary results. *Clin Physiol Funct Imaging*. 2016;36:499–503. doi: 10.1111/cpf.12254
41. Cocker MS, Spence JD, Hammond R, Wells G, deKemp RA, Lum C, Adeeko A, Yaffe MJ, Leung E, Hill A, et al; Canadian Atherosclerosis Imaging Network (CAIN). [18F]-NaF PET/CT identifies active calcification in carotid plaque. *JACC Cardiovasc Imaging*. 2017;10:486–488. doi: 10.1016/j.jcmg.2016.03.005
42. Forsythe RO, Dweck MR, McBride OMB, Vesey AT, Semple SI, Shah ASV, Adamson PD, Wallace WA, Kaczynski J, Ho W, et al. 18F-sodium fluoride uptake in abdominal aortic aneurysms: the SoFIA3 study. *J Am Coll Cardiol*. 2018;71:513–523. doi: 10.1016/j.jacc.2017.11.053
43. Chowdhury MM, Tarkin JM, Albaghdadi MS, Evans NR, Le EPV, Berrett TB, Sadat U, Joshi FR, Warburton EA, Buscombe JR, et al. Vascular positron emission tomography and restenosis in symptomatic peripheral arterial disease: a prospective clinical study. *JACC Cardiovasc Imaging*. 2020;13:1008–1017. doi: 10.1016/j.jcmg.2019.03.031
44. Nakahara T, Narula J, Tijssen JGP, Agarwal S, Chowdhury MM, Coughlin PA, Dweck MR, Rudd JHF, Jinzaki M, Mulhall J, et al. 18F-fluoride positron emission tomographic imaging of penile arteries and erectile dysfunction. *J Am Coll Cardiol*. 2019;73:1386–1394. doi: 10.1016/j.jacc.2018.10.076
45. Doris MK, Otaki Y, Krishnan SK, Kwiecinski J, Rubeaux M, Alessio A, Pan T, Cadet S, Dey D, Dweck MR, et al. Optimization of reconstruction and quantification of motion-corrected coronary PET-CT. *J Nucl Cardiol*. 2020;27:494–504. doi: 10.1007/s12350-018-1317-5
46. Lassen ML, Kwiecinski J, Dey D, Cadet S, Germano G, Berman DS, Adamson PD, Moss AJ, Dweck MR, Newby DE, et al. Triple-gated motion and blood pool clearance corrections improve reproducibility of coronary 18F-NaF PET. *Eur J Nucl Med Mol Imaging*. 2019;46:2610–2620. doi: 10.1007/s00259-019-04437-x
47. Jenkins WS, Vesey AT, Shah AS, Pawade TA, Chin CW, White AC, Fletcher A, Cartlidge TR, Mitchell AJ, Pringle MA, et al. Valvular (18)F-fluoride and (18)F-fluorodeoxyglucose uptake predict disease progression and clinical outcome in patients with aortic stenosis. *J Am Coll Cardiol*. 2015;66:1200–1201. doi: 10.1016/j.jacc.2015.06.1325