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Sodium fluoride: how an old marker gains a new meaning

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SODIUM FLUORIDE: HOW AN OLD MARKER GAINS A NEW MEANING

FLUORETO DE SÓDIO: COMO UM VELHO MARCADOR GANHA UM NOVO SIGNIFICADO

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ABSTRACT

Background. 18-Fluorine sodium fluoride is a well-known marker used for bone metastasis diagnosis. Its uptake correlation with cardiovascular risk was primarily suggested in oncological patients. Moreover, as a specific marker of microcalcification, it seems to correlate with cardiovascular disease progression and plaque instability.

Methods and Results. Our purpose was to systematically review clinical studies that characterized the use of this marker in cardiovascular conditions. In atherosclerosis, most studies report a positive correlation with the burden of cardiovascular risk factors and vascular calcification. A higher uptake was found in culprit plaques/rupture sites in coronary and carotid arteries and it was also linked to high-risk features in histology and intravascular imaging analysis of the plaques. In aortic stenosis, this tracer displayed an increasing uptake with disease severity.

Conclusions. Sodium fluoride positron emission tomography is a promising non-invasive technique to identify high-risk plaques, which sets ground to a potential use of this tracer in evaluating atherosclerotic disease progression and degenerative changes in aortic valve stenosis. Nevertheless, there is a need for further prospective evidence that demonstrates this technique value in predicting clinical events, adjusting treatment strategies and improving patient outcomes.

KEYWORDS

Positron emission tomography, sodium fluoride, cardiovascular risk, atherosclerosis, aortic valve stenosis

RESUMO

Introdução. O fluoreto de sódio é um conhecido marcador usado no diagnóstico de metástases ósseas. A correlação da captação do fluoreto de sódio com o risco cardiovascular foi primeiramente sugerido em doentes oncológicos. Adicionalmente, como marcador específico de microcalcificação, parece estar relacionado com a progressão da doença cardiovascular e instabilidade da placa aterosclerótica.

Métodos e Resultados. Fizemos revisão sistemática de estudos clínicos que caracterizaram o uso deste marcador em condições cardiovasculares. Na doença aterosclerótica, a maior parte dos estudos descrevem uma correlação positiva com os factores de risco cardiovasculares e calcificação vascular. Uma maior captação foi encontrada em placas responsáveis por eventos agudos/locais de ruptura coronária e carotídea, estando também ligado a características de alto risco em histologia e técnicas intravasculares de análise da placa. Na estenose aórtica, este marcador mostrou um aumento com a severidade da doença.

Conclusões. A tomografia de emissão de positrões é uma técnica não invasiva que permite a identificação de placas de alto risco, nomeadamente através do uso do fluoreto de sódio como marcador. Antevê-se o potencial deste marcador na avaliação da progressão da doença aterosclerótica e de alterações degenerativas na válvula aórtica. Contudo, a validação do uso clínico desta técnica ainda requer evidência prospectiva adicional que demonstre o seu valor na estratificação de risco, adequação de estratégias de tratamento e melhoria da sobrevida dos doentes.

PALAVRAS-CHAVE

Tomografia de emissão de positrões, fluoreto de sódio, risco cardiovascular, aterosclerose, estenose da válvula aórtica

ACRONYMS AND ABBREVIATIONS LIST / LISTA DE ACRÓNIMOS E ABREVIATURAS

^{18}F-NaF	Fluorine-18 sodium fluoride	Flúor-18 fluoreto de sódio
AAA	Abdominal aortic aneurysm	Aneurisma da aorta abdominal
AVS	Aortic valve stenosis	Estenose da válvula aórtica
CT	Computed tomography	Tomografia computorizada
CV	Cardiovascular	Cardiovascular
CVD	Cardiovascular disease	Doença cardiovascular
MI	Myocardial infarction	Enfarte do miocárdio
PET	Positron emission tomography	Tomografia de emissão de positrões

INTRODUCTION

Atherosclerotic cardiovascular (CV) events are the most common cause of death in developed countries. Clinical diagnosis of atherosclerotic CV disease (CVD) is usually late considering the atheroma pathogenesis process, mainly due to limitations in non-invasive diagnosis of this multi-stage disease.

Plaque rupture and/or erosion are the main causes of acute clinical events. From histologic and invasive clinical data, the culprit plaque tends to have a thin fibrous cap with scarce smooth muscle cells, positive remodelling, large lipid-rich cores, high macrophage content, luminal growing and microcalcifications.[1] In addition, degenerative aortic valve disease ultimately leads to hemodynamic stenosis of the valve with progressive leaflet thickening, dystrophic calcification and increased rigidity.[2] Therefore, microcalcification is one of the key markers of plaque's instability and degenerative changes in aortic valve stenosis (AVS). However, due to the small diameter of the formed calcic vesicles, they cannot be detected on routine computed tomography (CT), which only identifies macrocalcification (~200-500μm in diameter).[3]

There is a non-invasive marker of the microcalcification process. In fields such as oncology, fluorine-18 sodium fluoride (^{18}F-NaF) is a positron-emitting radiotracer used for the study of bone metabolism pathologies and bone metastasis.[4] The high linkage affinity between fluoride ions and the surface of calcic hydroxyapatite represents the main biochemical principle for the identification of osteoblastic activity and new bone formation.[5] The pattern of this tracer's uptake depends on differences in regional blood flow, the density of hydroxyapatite as well as exposed crystal surface area. ^{18}F-NaF binds avidly to microcalcification while areas of macrocalcification show only peripheral uptake thanks to its large volume (low surface-area-to-volume ratio).[3,6,7]

This systematic review aims to summarize and consolidate ^{18}F-NaF potentialities to study CVD using positron emission tomography (PET).

METHODS

We systematically reviewed clinical studies with prospective/retrospective methodology that looked into the use of ^{18}F-NaF in CV conditions. All the *in vivo* clinical trials made for CV purposes that correlated ^{18}F-NaF activity with atherosclerotic disease, AVS and abdominal aortic aneurysms (AAA), met the inclusion criteria. Only full original scientific articles were included. We excluded any studies that did not meet all the inclusion criteria. Additionally, we excluded trials only aiming to determine technical protocols for image acquisition using ^{18}F-NaF uptake.

In July of 2018, three separate searches were conducted for each group of pathologies and their correlation with ^{18}F-NaF, using PubMed, Embase and Cochrane Library. The MeSH, Emtree and other additional terms “sodium fluoride”, “18f naf”, “18f fluorine”, “fluorine 18”, “f naf”, “atherosclerosis”, “atheroma”, “atherosclerotic plaque”, “aortic valve stenosis”, “aortic stenosis”, “aortic valve disease”, “valvular heart disease” and “aortic aneurysm” in various combinations were used to build the research.

If relevant, additional articles were added to the screened records.

DATA COLLECTION

In the selected studies, the information concerning the following parameters was collected: 1) imaging sites evaluated; 2) design/study type; 3) sampling inclusion criteria; 4) number of patients; 5) injected *in vivo* dose of ^{18}F-NaF measured in megabecquerel (MBq); 6) uptake period from intravenous administration to image acquisition; 7) study aim; 8) number of patients with 18F-NaF uptake and positivity criteria; 9) results regarding ^{18}F-NaF.

SUMMARY MEASURES

Target-to-background ratio (TBR) is calculated dividing the maximum standardized uptake value (SUV_{max}) with blood pool SUV. Correspondently, SUV_{max} consists in the division of the decay corrected tissue concentration of the tracer and the injected dose per bodyweight; blood pool SUV is estimated as the mean of five regions of interest (ROI) in the mid lumen of superior vena cava. Corrected uptake per lesion (CUL) can also be used, subtracting the blood pool SUV from the SUV_{max}.

RESULTS

On the basis of the presented criteria, 31 articles were reviewed (Appendix I).

ATHEROSCLEROSIS

Twenty-five clinical studies looked into the use of ^{18}F-NaF in the setting of atherosclerosis (Table 1). In this matter, arterial wall ^{18}F-NaF uptake was primarily assessed in oncological patients that underwent PET/CT imaging. The majority of these retrospective studies showed a positive correlation of this marker with calcification and the number of CV risk factors.[8-14] For instance, in a retrospective analysis of over 400 patients by Janssen et al., ^{18}F-NaF uptake in the femoral arteries increased with the amount of CV risk factors and the calcified plaques per patient.[12] Furthermore, baseline ^{18}F-NaF uptake significantly correlated with calcification progression and Agaston score in a CT performed one year after.[14]

Prospective studies were subsequently carried out on populations that met CV inclusion criteria or with known CVD. Four studies performed in patients with different risk profiles demonstrated that ^{18}F-NaF uptake increases with the number of CV risk factors.[15-18] In a prospective analysis of 89 healthy subjects with low CV risk, Blomberg et al. showed that the estimated future risk for CVD development increases linearly with coronary ^{18}F-NaF accumulation.[17] Our group studied patients with high CV risk, examining ^{18}F-NaF uptake in the major arterial territories (coronary, aorta, carotid), and found similar correlations with risk factors, including thoracic fat volume.[15,16] Other five studies looked into ^{18}F-NaF uptake in patients with known/suspected CVD and showed that this marker is able to identify high-risk atherosclerotic plaques and culprit lesions.[7,19-22] Within this group, Dweck et al. conducted the first feasibility study on the coronary arteries and found a higher uptake in patients with coronary atherosclerosis.[19] Later, Joshi et al. prospectively studied the coronary and carotid arteries of patients with myocardial infarction (MI), stable angina and symptomatic carotid artery disease.[7] In this landmark report, 37 out of 40 patients with MI had higher uptake in culprit plaques, which were 34% more intense than non-culprit sites.[7] Moreover, superior ^{18}F-NaF uptake was linked to high-risk features identified in endarterectomy specimens histology.[7] Subsequently, Lee et al. used intravascular ultrasonography and optical coherence tomography techniques in stable coronary heart disease patients to validate these findings, correlating ^{18}F-NaF uptake with high-risk features assessed *in vivo*.[21] Remarkably, 93% of the lesions that met high-risk criteria in both techniques showed ^{18}F-NaF uptake.[21]

In cerebrovascular disease population, symptomatic and culprit carotid lesions also showed a significant correlation with ^{18}F-NaF uptake.[6,23,24] The use of advanced techniques (histological marking and micro PET/CT) supported previous clinical observations, showing a

strong correlation of ^{18}F-NaF uptake with calcification markers (alizarin red staining) and with clinically symptomatic plaques.[6,25]

The concept of micro- and macrocalcification differentiation was studied by Irkle et al. in a complementary *in vivo* and *ex vivo* analysis.[3] Firstly, *in vivo* PET imaging in symptomatic patients scheduled to undergo carotid endarterectomy explored the pharmacodynamic and pharmacokinetic properties of ^{18}F-NaF, where this tracer showed conducive imaging properties with excellent tissue contrast resolution.[3] Besides, cellular specificity of ^{18}F-NaF binding was histologically correlated with markers of calcification, but not with macrophages, neovascularization and smooth muscle markers.[3] Moreover, ^{18}F-NaF signal was compared with sectioned carotid tissue micro- and macrocalcification in μPET/μCT, with its binding increased in microcalcifications (<50μm) and detected only on the outer surface layer of macrocalcific deposits.[3]

AORTIC STENOSIS

Four clinical studies looked into the use of ^{18}F-NaF in AVS (Table 2). The first feasibility study concluded that there is an intense uptake of this marker in patients with known AVS.[26] Further reports demonstrated ^{18}F-NaF ability to mark different disease stages and described this tracer's accumulation in proportion with calcium scores, blood and histological markers of calcification and disease progression.[27-29] In fact, ^{18}F-NaF uptake was associated with disease severity and correlated with histological markers of active calcification.[27-29] Dweck et al. reported a positive uptake (TBR_{max}>1.97) in 100% of severe, 95% of moderate and 76% of mild AVS.[27] ^{18}F-NaF uptake was also a good predictor of disease progression by CT calcium score after one year, with concordant distribution of new calcic deposits in regions with previous radiotracer accumulation.[29]

OTHER APPLICATIONS

In a study by Forsythe et al., ^{18}F-NaF uptake was increased in AAA compared to non-aneurysmal regions within the same aorta and co-localized with areas of aneurysmal disease activity and active calcification.[30] Moreover, ^{18}F-NaF uptake is likely a major predictor of aneurysm expansion (over twice the expansion rate in the highest tertile of uptake), showing three times higher risk of repair or rupture in the highest tertile of uptake.[30]

A small clinical study assessed whether ^{18}F-NaF could determine Takayasu arteritis disease grade and evaluate its progression.[31] There was a correlation between ^{18}F-NaF uptake and different stages of metabolic disease.[31]

In patients with recent MI, ^{18}F-NaF accumulation was significantly higher in scar tissue than in healthy myocardium.[22]

Table 1 – Clinical studies of atherosclerosis and ^{18}F-NaF

Study/Authors	Year*	Site	Design/Study Type	Inclusion Criteria	Patient Number	Dose (MBq)	Uptake Period (min)	Aim	Primary Outcome Measure/Prevalence (Patients)	Results
ONCOLOGICAL POPULATIONS										
Derlin et al.[8]	2010	Major arteries	Retrospective	Previous ^{18}F-NaF PET/CT for bone metastases	75	350±50	60	Arterial ^{18}F-NaF uptake correlation with calcification	Segments with uptake, regardless the intensity/57 (76%)	88% of the lesions with ^{18}F-NaF uptake showed calcification.
Beheshti et al.[32]	2011	Heart and aorta	Retrospective	Previous ^{18}F-NaF PET/CT for malignancy	51	370-550	60	Degree and prevalence of ^{18}F-NaF uptake as calcification evidence in atherosclerotic plaques with normal aging	Segments with uptake, regardless the intensity/Not described	Cardiac and aortic ^{18}F-NaF uptake and degree of calcification correlated significantly with age.
Derlin et al.[9]	2011	Major arteries	Retrospective	Previous ^{18}F-NaF PET/CT in oncologic patients	45	350±50	60	Comparison between ^{18}F-NaF and ^{18}F-FDG uptake and correlation with CPB	Segments with uptake, regardless the intensity/27 (60%)	77.1% of the lesions with ^{18}F-NaF uptake showed calcification.
Derlin et al.[10]	2011	Carotid arteries	Retrospective	Previous ^{18}F-NaF PET/CT for bone metastases	269	350±50	60	Correlation between ^{18}F-NaF uptake in the common carotid arteries, CV risk factors and CPB	Segments with uptake, regardless the intensity/94 (34.9%)	^{18}F-NaF uptake and calcification co-localized at all sites and were highly correlated. High correlation between ^{18}F-NaF uptake and the number of CV risk factors, age, male sex, hypertension and hypercholesterolemia.
Li et al.[11]	2012	Major arteries and coronary arteries	Retrospective	Previous ^{18}F-NaF PET/CT for bone studies	61	370±74	40	Vascular ^{18}F-NaF uptake association with vascular calcification and CHD	TBR≥1.5 in all orthogonal image planes/59 (97%)	^{18}F-NaF uptake positively correlated with calcification and history of CV events. Neither the individual CV risk estimation nor the number of risk factors correlated with coronary uptake.
Janssen et al.[12]	2013	Femoral arteries	Retrospective	Previous ^{18}F-NaF PET/CT in oncologic patients	409	350±50	60	Linear ^{18}F-NaF uptake correlation with CV risk factors and CPB	Uniform linear railroad track-type tracer uptake >two-thirds the vessel's length/159 (38.9%)	Significant correlation between ^{18}F-NaF uptake and age, hypertension, hypercholesterolemia, diabetes, history of smoking, prior CV events and CPB. Linear ^{18}F-NaF uptake increased with the number of CV risk factors and the number of calcified plaques per patient.
Kurata et al.[13]	2013	Major arteries	Retrospective	Oncologic patients with ^{18}F-NaF PET/CT for bone metastases	29	≈185	60	Evaluation of ^{18}F-NaF uptake prevalence, distribution, and relationship with arterial calcification	Segments with uptake, regardless the intensity/8 (28%)	91% of the lesions with ^{18}F-NaF uptake showed calcification. Only 10% of the calcified lesions showed increased ^{18}F-NaF uptake. Arterial uptake and calcification significantly associated with age.
Morbelli et al.[33]	2014	Major arteries	Prospective	Oncologic patients undergoing ^{18}F-NaF PET/CT for bone metastases	80	370	60	^{18}F-NaF uptake correlation with Framingham risk factors and CPB	Segments with uptake, regardless the intensity/Not described	^{18}F-NaF uptake significantly correlated with age, hypertension, smoking and diabetes. Visible calcium deposition depended only on age. No correlation between regional calcium load and TBR.

Fiz et al.[34]	2015	Infrarenal abdominal aorta	Prospective	Oncologic patients undergoing ^{18}F-NaF PET/CT for bone metastases with ≥1 arterial calcification in the infrarenal abdominal aorta	64	4.8-5.2/kg of body weight	60-75	^{18}F-NaF uptake evaluation in different plaque density calcification and whether attenuation weighted image reconstruction affected ^{18}F-NaF uptake values in the different plaque stages	Segments with uptake, regardless the intensity/Not described	Inverse correlation between ^{18}F-NaF uptake and density of calcification.
Ishiwata et al.[14]	2017	Major arteries	Retrospective	Malignancy or orthopaedic disease patients with whole-body ^{18}F-NaF PET/CT and an unenhanced CT approximately 1 year after admission	34	185	40	Vascular ^{18}F-NaF uptake correlation with CT calcium score progression	1.5 SUVmax cut-off/Not described	^{18}F-NaF uptake significantly correlated with calcification progression, volumetric score and progression in Agaston score.
Li et al.[35]	2017	Major arteries	Retrospective	Multiple myeloma patients with ^{18}F-NaF PET/CT for bone metastases	34	310-380	Not described	^{18}F-NaF and ^{18}F-FDG uptake association with plaque density calcification and disease progression	1.8 TBR for positive osteogenesis/Not described	Higher ^{18}F-NaF uptake in non-calcified and severely calcified lesions and lower uptake in mildly calcified plaques. During follow-up, ^{18}F-NaF uptake increased with calcium density.
SUBCLINICAL ATHEROSCLEROSIS										
Dweck et al.[19]	2012	Coronary arteries	Prospective	Control subjects and patients aged >50 years with aortic sclerosis and mild, moderate and severe AVS	119	124±10	66±6	^{18}F-NaF and ^{18}F-FDG PET/CT uptake as markers of active calcification and inflammation, respectively	TBRmax>1.45 (higher control group value)/34%	Higher ^{18}F-NaF uptake in patients with coronary atherosclerosis. Strong correlation between the CCS and ^{18}F-NaF uptake, although 41% of patients with CCS>1,000 had no significant uptake. Higher Framingham risk scores in patients with increased ^{18}F-NaF uptake. 10 year Framingham risk scores for CVD, CVD death and CHD death correlated with ^{18}F-NaF coronary uptake but not with CCS.
Ferreira et al.[15]	2017	Carotid arteries, coronary arteries and aorta	Prospective	Asymptomatic patients aged >40 years with high CV risk without known CVD	25	2.5/kg of body weight (185)	60	^{18}F-NaF uptake evaluation within the arterial wall in high CV risk patients	Segments with uptake, regardless the intensity/24 (96%)	TBR but not CUL is higher in men than in women while CUL but not TBR is related to the number of CV risk factors.
Oliveira-Santos et al.[16]	2017	Carotid arteries, coronary arteries and aorta	Prospective	Asymptomatic patients aged >40 years with high CV risk without known CVD	25	185	60	Correlation of ^{18}F-NaF uptake in high CV risk participants with CV risk factors, CCS and thoracic fat volume	Segments with uptake, regardless the intensity/96% in aorta; 40% in carotid arteries; 64% in coronary arteries	Patients with ≥5 risk factors (60%) had increased ^{18}F-NaF uptake; the latter was positively correlated with predicted fatal CV risk. No correlation between ^{18}F-NaF uptake in coronary arteries and calcium score. Moderate correlation between CUL and thoracic fat.

Blomberg et al.[17]	2017	Coronary arteries	Prospective	Healthy subjects with low CV risk	89	2.2/kg of body weight (174±39)	92±4	Coronary artery ^{18}F-NaF uptake correlation with CV risk	Segments with uptake, regardless the intensity/Not described	In healthy adults, sex, age and BMI are independent determinants of coronary ^{18}F-NaF uptake. Coronary ^{18}F-NaF uptake increased linearly with the number of CV risk factors. The estimated 10- and 30-year risk of CVD increased linearly with the coronary ^{18}F-NaF uptake.
Blomberg et al.[18]	2017	Thoracic aorta	Prospective	Low CVD risk population: patients with chest pain syndromes and healthy volunteers	139	2.2/kg of body weight (174±35)	91±4	Comparison between ^{18}F-NaF and ^{18}F-FDG uptake and correlation with vascular calcium burden	Segments with uptake, regardless the intensity/Not described	^{18}F-NaF uptake positively correlated with CT calcium burden and higher CVD risk.
CORONARY ARTERY DISEASE										
Joshi et al.[7]	2014	Coronary and carotid arteries	Prospective	Patients with pre-established MI or stable angina and patients undergoing carotid endarterectomy for symptomatic disease	89	123	60	Ruptured and high-risk atherosclerotic plaques identification using ^{18}F-NaF and ^{18}F-FDG	TBR>25% than a proximal reference lesion/37 of 40 (93%) with MI in the culprit plaque; 18 of 40 (45%) with stable CHD	93% MI culprit plaques showed increased ^{18}F-NaF, 34% higher in culprit plaques than the maximum activity anywhere else in the coronary vasculature. High ^{18}F-NaF uptake in all carotid plaque rupture sites which had higher calcification activity, macrophage infiltration and cell death. ^{18}F-NaF uptake in plaques of stable angina patients was mostly non-obstructive (<70% stenosis) and had high-risk features: positive remodelling, microcalcification and necrotic core.
Kitagawa et al.[20]	2017	Coronary arteries	Prospective	Known or suspected CHD with ≥1 coronary atherosclerotic lesion detected on CCTA in segments >2mm in diameter	32	370	60	^{18}F-NaF PET/CT uptake correlation with CT coronary atherosclerosis	For each atherosclerotic lesion, a ROI was drawn in a proximal reference site without plaque to calculate the control TBRmax/Not described	CCS correlated positively with coronary ^{18}F-NaF uptake. MI or unstable angina history and coronary plaques with CCTA-identified high-risk features showed higher coronary ^{18}F-NaF uptake.
Lee at al.[21]	2017	Coronary arteries	Prospective	Suspected CHD patients with ^{18}F-NaF PET prior to invasive coronary angiography	51	3/kg of body weight	60	^{18}F-NaF PET/CT clinical relevance evaluation using OCT, IVUS and CCTA in patients with CHD	Plaques with uptake, regardless the intensity/Not described	Plaques with ^{18}F-NaF uptake showed higher plaque burden, more frequent posterior attenuation and positive remodelling in IVUS and higher maximum lipid arc and more microvessels in OCT. 94.1%^{18}F-NaF positive lesions had ≥1 high-risk features. 93.3% high-risk lesions showed ^{18}F-NaF uptake.
Marchesseau et al.[22]	2017	Coronary arteries and myocardial tissue	Prospective	STEMI patients undergoing primary percutaneous coronary intervention	10 PET/MR; 8 PET/CT	PET/MR: 2.95±0.21 mCi; PET/CT: not described	PET/MR: 60-75; PET/CT: 108±21	^{18}F-NaF PET/CT validation for the detection of high-risk coronary atheroma and PET/MR quantification of the uptake in myocardial healthy and scar tissue	1.70 TBR threshold to high and low-risk lesions/Not described	TBR of culprit lesions higher than non-culprit. TBR higher in scar tissue than in healthy myocardium.

CEREBROVASCULAR DISEASE										
Quirce et al.[23]	2013	Carotid arteries	Prospective	Patients with ≥1 plaque detected by contrast CT during a neurological work-up	15	370	180	^{18}F-NaF uptake correlation and identification in carotid arteries and previously identified calcified plaques	Plaques with uptake, regardless the intensity/100%	All plaques revealed some degree of uptake. The plaques of the symptomatic group had higher uptake.
Irkle et al.[3]	2015	Carotid atheroma	Prospective	Patients undergoing carotid endarterectomy for symptomatic stenosis	4	250	60	Active vascular microcalcification identification by ^{18}F-NaF PET/CT	Not described	Higher uptake regions in the absence of calcium on CT, representing microcalcification (<50µm). Large CT-detected areas of macrocalcification without uptake. Higher ^{18}F-NaF activity on the surface of macroscopic calcic deposits and not within their core.
Quirce et al.[24]	2016	Carotid atheroma	Prospective	Recent stroke	9	370	180	Comparison between ^{18}F-NaF and ^{18}F-FDG uptake in symptomatic and non-symptomatic plaques	Plaques with uptake, regardless the intensity/100%	^{18}F-NaF uptake higher than ^{18}F-FDG in 11 of the 18 plaques. Higher mean TBR in the symptomatic group. In symptomatic plaques, ^{18}F-NaF uptake showed an inverse correlation with calcium burden.
Vesey et al.[6]	2017	Carotid atheroma	Prospective	Recent transient ischemic attack or minor ischemic stroke: culprit carotid stenosis awaiting endarterectomy and controls without culprit carotid atheroma	26	244.5±12.66	64.5±5.6	Comparison between ^{18}F-NaF and ^{18}F-FDG uptake ability to identify culprit and high-risk carotid plaques	Plaques with uptake, regardless the intensity/Not described	On histological and micro PET/CT analysis, ^{18}F-NaF selectively identified microcalcification. Higher ^{18}F-NaF uptake in clinically adjudicated culprit plaques. ^{18}F-NaF uptake correlated with high-risk plaque features, plaque burden and predicted CV risk.
Zhang et al.[25]	2018	Carotid arteries	Prospective	Symptomatic patients undergoing carotid endarterectomy for carotid artery stenosis (<70%)	8	4.44/kg of body weight	60	^{18}F-NaF uptake correlation with histological characterization of vascular calcification in carotid plaques	Segments with uptake, regardless the intensity/100% in the bilateral carotid bifurcation	Strong correlation between ^{18}F-NaF uptake and alizarin red staining - calcification marker. No correlation with CD68 staining -inflammation marker- and alpha-smooth muscle actin staining - smooth muscle marker. No correlation with carotid artery stenosis, HU value or inflammation.

^{18}F-NaF, fluorine-18 sodium fluoride; ^{18}F-FDG, fluorine-18 fluoro-deoxy-glucose; CCS, coronary calcium score; CCTA, coronary CT angiography; CHD, coronary heart disease; CPB, calcified plaque burden; CT, computed tomography; CUL, corrected uptake per lesion; CV, cardiovascular; CVD, cardiovascular disease; HU, Hounsfield unit; IVUS, intravascular ultrasound; mCI, millicurie; MI, myocardial infarction; MR, magnetic resonance; OCT, optical coherence tomography; PET, positron emission tomography; ROI, region of interest; STEMI, ST-elevation myocardial infarction; SUV, standardized uptake value; TBR, target to background ratio.
*Publication date

Table 2 – Clinical studies of aortic valve stenosis and ^{18}F-NaF

Study/Authors	Year*	Site	Design/Study Type	Inclusion Criteria	Patient Number	Dose (MBq)	Uptake Period (min)	Aim	Primary Outcome Measure/Prevalence (Patients)	Results
Hyafil et al.[26]	2012	Aortic valve	Retrospective	Severe AVS patients and patients free of aortic valvular calcium on CT, with ^{18}F-NaF PET/CT for oncologic or rheumatologic purposes	15	4/kg of body weight	40	^{18}F-NaF accumulation in degenerative aortic valves	Higher TBR values than the control group/100% with severe AVS; 0% free of valvular calcium	^{18}F-NaF accumulated in calcified valves. All patients with known AVS had intense ^{18}F-NaF accumulation on previous PET/CT. Patients free of valve calcium had no significant ^{18}F-NaF accumulation.
Dweck et al.[27]	2012	Aortic valve	Prospective	Control subjects and patients aged >50 years with aortic sclerosis and mild, moderate and severe AVS	121	124±10	66±7	^{18}F-NaF and ^{18}F-FDG uptake comparison in different stages of aortic stenosis	TBRmax >1.97 (higher control group value)/0% control patients, 45% with aortic sclerosis and 91% with AVS (76% mild; 95% moderate; 100% severe)	^{18}F-NaF uptake higher in patients with aortic sclerosis and stenosis. ^{18}F-NaF activity increased with disease severity. All measures of ^{18}F-NaF uptake correlated with increasing aortic jet velocity, valve calcium score and other echocardiographic measures of AVS severity.
Dweck et al.[28]	2013	Aortic valve, coronary arteries, thoracic aorta and bone	Prospective	Patients aged >50 years with aortic sclerosis and mild, moderate and severe AVS	101	125±10	66±6	^{18}F-NaF and ^{18}F-FDG uptake comparison in aortic valve calcification with that measured in atherosclerosis and bone	Increased ^{18}F-NaF activity compared with background/100%	Weak/absent correlations between calcium scores and calcific atheroma, as between calcium scores and bone mineral density. Valvular ^{18}F-NaF activity higher than in the aorta and correlated strongly with AVS severity. No clinically significant correlations with blood markers of calcium metabolism.
Dweck et al.[29]	2014	Aortic valve	Prospective	AVS	30	120-126	60	^{18}F-NaF and ^{18}F-FDG uptake comparison with aortic valve histological characterization and prediction of disease progression	TBRmax>1.97/100%	^{18}F-NaF uptake correlated with histological markers of active calcification (TNAP and osteocalcin) and was a good progression predictor in valve CT calcium scores at 1 year.

^{18}F-NaF, fluorine-18 sodium fluoride; ^{18}F-FDG, fluorine-18 fluoro-deoxy-glucose; AVS, aortic valve stenosis; CT, computed tomography; PET, positron emission tomography; TBR, target to background ratio; TNAP, tissue nonspecific alkaline phosphatase.
*Publication date

DISCUSSION

By targeting active microcalcification areas, ^{18}F-NaF PET has enhanced the study of CVD, with potential staging of different lesion types and assessment of disease progression.

In this systematic review we aimed to resume the emerging data related to the use of ^{18}F-NaF in CV fields. There is an association between ^{18}F-NaF uptake and CV risk factors and also with high-risk patients. Such findings suggest that measurements of this tracer's accumulation are associated to plaque instability and AVS disease progression. The integration of overall risk assessment with lesion characteristics, by non-invasive molecular imaging, may be valuable and allow further insights into accurate individual risk prediction, along with tailored primary/secondary CVD prevention interventions (antiplatelets, high intensity lipid lowering therapies, etc.).

^{18}F-NaF proved to be of value to the identification of plaque characteristics, but we have yet to clarify the clinical potential of this analysis. Are we really identifying the unstable plaque, the holy grail of preventive cardiology? Despite major advances in atheroma fine characterization, from different invasive intravascular imaging modalities and coronary CT, the medical community gradually lost some enthusiasm in the appealing concept of vulnerable plaque. In fact, even though the evidence from PROSPECT demonstrated that some plaque characteristics increase the probability of event, it is still more likely to have complications in other territories, due to a higher plaque number.[36] Over time, the focus shifted to the "vulnerable patient" thesis, with a comprehensive analysis of several risk factors in order to address overall CVD prevention.[37] Indeed, the multivariable analysis of a recent large study showed that the higher risk of CV events conferred by the presence of both obstructive disease and adverse plaque features was not independent from coronary calcium score (CCS).[38] When compared to CCS, carotid arteries ultrasound or risk estimation employing clinical calculators, ^{18}F-NaF molecular imaging has the potential to non-invasively identify high-risk plaques in several vascular territories, conveying anatomical and metabolic data, thus entailing a broader measure of CV risk stratification. However, this hypothesis requires testing in large longitudinal studies.

AVS is the most common valvular disease in developed countries, with rising prevalence in parallel with ageing. Its progression is not yet completely understood and therapeutic/preventive measures still remain a challenge with underlying scarce disease-modifying results. Initial data shows that ^{18}F-NaF may predict disease activity and progression, thus being a valuable tool both to the assessment of AVS and the documentation of novel therapies' effect, without the need for extensive follow-up periods with contemporary imaging techniques.

The emerging data on ^{18}F-NaF imaging has several limitations. Many studies used small sample size with limited external validity, such as oncological populations. Furthermore, there is no agreement in scanning methodology with inhomogeneous measures and cut-off criteria ("significant/positive uptake" classification). PET/CT limitations in spatial resolution can hamper the visualization of small vessels and the patient's radiation exposure is not negligible. For the latter, newer machines with magnetic resonance can lower the radiation dose.

In conclusion, there is mounting evidence on the utility of ^{18}F-NaF PET imaging to identify atheroma instability and CVD progression. Future potential uses of this technology include CV risk stratification, selection of patients eligible to intensive CV prevention strategies, identification of culprit plaques in multivessel disease, or even to serve as a surrogate endpoint for CVD trials. However, further prospective clinical studies are necessary to better define the role of ^{18}F-NaF uptake, standardization of the imaging acquisition methodology and measurement tools.

NEW KNOWLEDGE GAINED

There is growing evidence on ^{18}F-NaF PET molecular imaging potentialities, namely in the identification of high-risk atheroma plaque, high-risk CV patients and aortic stenosis progression. Microcalcification identification is an emergent field in the study of CVD.

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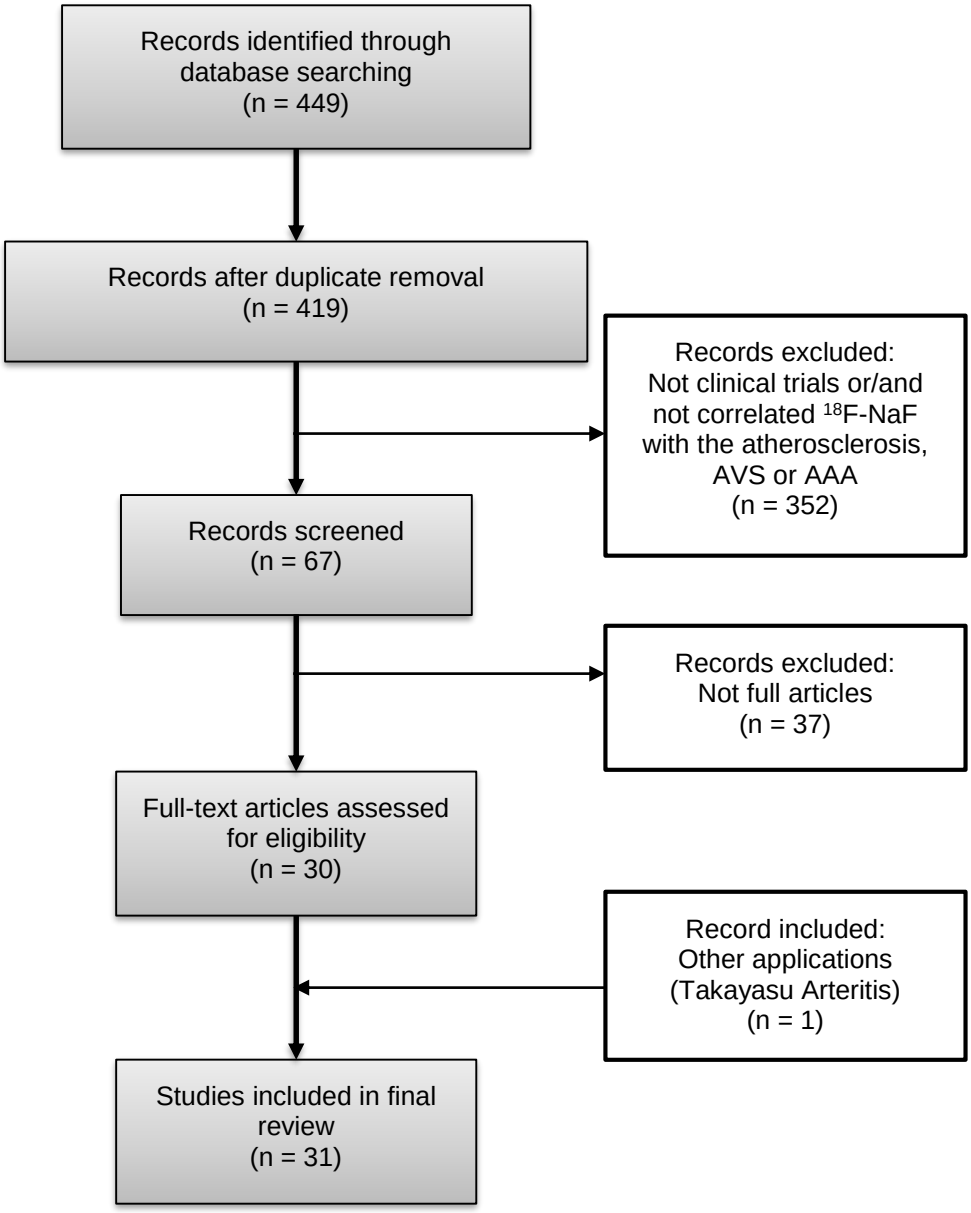
APPENDIX I – Results of search strategy diagram



^{18}F-NaF, sodium fluoride; AAA, abdominal aortic aneurysm; AVS, aortic valve stenosis.