18F-NaF uptake by the atherosclerotic plaque at PET/CT imaging: inverse correlation between calcification density and mineral metabolic activity

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ABSTRACT

Several studies highlighted the role of vascular $^{18}$F-NaF uptake as a marker of ongoing calcium deposition. However, $^{18}$F-NaF accumulation often shows inconsistent co-localization with arterial plaque. Actually, calcification activity and thus $^{18}$F-NaF uptake could prevail in the earlier plaque stages. To test this hypothesis, we evaluated $^{18}$F-NaF uptake in three plaque types using plaque density as a marker of calcification progression. We also tested whether attenuation weighted image reconstruction affected $^{18}$F-NaF uptake values in the different plaque stages considered.

**Methods:** 64 oncologic patients (14 males, mean age 65.3±8.2 range 26-81) underwent $^{18}$F-NaF PET/CT. A volume of interest (VOI), was drawn on each plaque within the infra-renal aorta, to assess mean SUV and attenuation value (HU). Plaques were then divided in light (LP, HU <210), medium (MP, HU 211 to 510) and heavy (HP, HU >510). SUV was normalized for blood $^{18}$F-NaF activity, to obtain plaque target-to-background ratio (TBR). In this process, several focal, non-calcified $^{18}$F-NaF uptake areas were identified (hot spots, HS). TBR was computed in HS, after iso-contour thresholding. TBR was also calculated in a non-calcified control region (CR). In 35 patients, TBR was furthermore calculated on non-attenuation-corrected (NAC) images.

**Results:** Among plaques, average TBR was highest in LP (2.21±0.88), while it was significantly lower in MP (1.59±0.53, p<0.001) and further decreased in HP (1.14±0.37, p<0.0001 with respect to both LP and MP). CR TBR was not significantly different to the one of HP and MP (p=ns), while it was significantly lower than LP and MP (p<0.01). HS had the highest absolute TBR (3.89±1.87, p<0.0001 vs LP). Considering TBR values originated from NAC didn’t provide significant difference from AC.

**Conclusion:** The present study is in keeping with the concept that $^{18}$F-NaF is a feasible option in imaging molecular calcium deposition in the early stages of plaque formation, when active uptake mechanism are the main determinants of calcium presence while its retention progressively decreases with increasing calcium deposition in the arterial wall. From a technical point of view, our data suggest that NAC reconstruction doesn’t significantly affect output when evaluating plaques of any thickness.

**Key Words:** $^{18}$F-Natrium Fluoride; Plaque Imaging, PET/CT
INTRODUCTION

Since the earliest pathological studies, arterial calcifications (AC) have been regarded as focal areas of ectopic formation of skeletal-like tissue, caused by continuous calcium deposition in the arterial intima (1). Modern hystopathological research has confirmed this hypothesis, demonstrating the presence of cells deriving from osteoblastic and osteoclastic lineage in areas of vascular calcification (2,3). These characteristics allowed for the use of X-ray computed tomography (CT) in the assessment of AC presence and to generate a clinical calcification score, which is widely regarded as a sound index of burden and extension of the atherosclerotic plaque (4,5). However, quantification of calcium score represents a static picture of calcific burden, which is unable to depict the pathophysiological behavior of the plaque and may not fully correlate with patients’ clinical status and cardiovascular risk (6-8). Therefore, on the basis of the aforementioned similarities between AC formation and osteogenesis, functional studies with 18F-sodium Fluoride (18-F NaF) positron emission tomography (PET) have been carried out, in the effort of complementing the calcific burden quantification with functional data on plaque mineral metabolism (9-15). These studies however highlighted a striking discrepancy between PET and CT plaque imaging features. In particular PET-positive areas were co-localized with AC almost invariably, while only a smaller fraction of AC showed visible 18F-NaF uptake (9). Moreover, the fraction of AC-correlated tracer uptake was widely variable in relation to the arterial segment studied, being as low as 12% if the average of all calcification sites was considered (9), showing great variability in the coronary district (15) and peaking in the carotid arteries (10).

The relevant mismatch between morphological and radioisotope findings might be grounded on pathophysiological considerations of plaque build up, which is a complex and dynamic process, involving repeated sequences of inflammation, repair, apoptosis and necrosis. Arterial
Calcification is however a prominent feature of atherosclerosis and is thus utilized as an imaging marker of plaque progression (9,10). In the earliest calcification phases, the inflammatory environment fosters the release of several bone-forming peptides, which promote the active apposition of a hydroxyapatite matrix in the arterial wall, appearing at CT imaging as “soft”, inconspicuous and sparse areas calcification (3). Conversely, in the more advanced phases AC appears as a continuous, clumped and dense area of calcification, sometimes completely encircling the vessel; this aspect indicates the quiescent stage of calcification, with further calcium apposition occurring only as the wall becomes progressively unable to support the homeostatic mechanisms that prevent extracellular calcium precipitation (16). While early and late stages may show a certain degree of overlap in some plaques, these two phases are mostly metachronous. Tracer features of 18F-NaF are actually well suited to image the early calcification phase, but plaque characteristics in the late phase could prevent any radioisotope approach (13). Moreover, a confounding contribution could be brought upon by a systematic overcorrection artifact, caused by the influence of high-density calcium clumps on the attenuation correction reconstruction of PET raw dataset (17).

The present study was thus planned to assess whether the evolutionary AC stage has an influence on plaque tracer uptake. In doing so, we hypothesized that plaques that are still in their growth process and for this reason are relatively unapparent on CT images have a higher 18F-NaF uptake, since in this stage active mechanisms of calcium deposition prevail. Conversely, we postulated that end-stage calcification do not present increased values of tracer uptake. To complete this evaluation, we also challenged the influence of attenuation-corrected reconstruction on AC uptake values.
MATERIALS AND METHODS

Patients

The study included 64 patients with either breast or prostate cancer (14 males, mean age 58.7±10.4 range 26-81) undergoing $^{18}$F-NaF PET/CT scan for evaluation of presence of bone metastases. Inclusion criteria comprised the presence of at least one AC in the infarenal abdominal aorta. AC was defined as a mural area, having a minimum HU of 130 (14). Exclusion criteria included history of vasculitis, autoimmune or systemic inflammatory disease as well as chemo- or radiotherapy in the preceding 8 weeks, as previously proposed (18). Ongoing or previous statin treatment was used as a further exclusion criterion to exclude the influence of these drugs on the results (19).

Written informed consent was obtained from each patient before the exam. The Institutional Ethics Committee approved this retrospective study and the requirement to obtain specific informed consent for research purposes was waived.

$^{18}$F-NaF PET acquisition and reconstruction

Patients underwent 18F-NaF PET/CT using two 16 slices PET/CT hybrid systems: 1) Biograph 16 (Siemens Medical Solutions, Knoxville TN, USA); 2) Discovery LS (GE Medical Systems, Milwaukee, WI, USA).

In both cases patients received an intravenous bolus injection of 18F-NaF (4.8-5.2 MBq per kilogram of body weight). PET/CT acquisition started 60-75 minutes thereafter, in the meantime the patient was hydrated and encouraged to void, as to diminish the unbound tracer fraction. The entire body was scanned from vertex to toes in an “arms down” position; emission scan lasted 120” per bed position. PET raw data were reconstructed by means of ordered subset expectation maximization (OSEM, 3 iterations, 16 subsets) and attenuation correction was performed using
CT data. Both attenuation corrected (using CT raw data) and non-attenuation corrected PET data were obtained. The transaxial field of view and pixel size of the reconstructed PET images were 58.5 cm and 4.57 mm, respectively, with a matrix size of 128×128. As per standard PET/CT imaging protocol, 16-detector row helical CT scan was performed with non-diagnostic current and voltage settings (120 Kv, 80 mA), with a gantry rotation speed of 0.5 s and table speed of 24 mm per gantry rotation. No contrast medium was injected. The entire CT dataset was fused with the 3-dimensional PET images using an integrated software interface (Syngo; Siemens Erlangen, Germany). Low dose CT (reconstructed at 4mm thick slices) was used for anatomical reference for the localization of vascular calcification.

Image Analysis

ACs were identified within the walls of the infrarenal abdominal aorta, defined as the segment comprised between the emergence of the renal arteries and the iliac carrefour. This vessel was selected as it shows a high prevalence of different types of calcification, both initial and end-stage, in the general population (20). ACs were excluded from the analysis if there was considerable suspect of spillover from a nearby structure (e.g. lumbar vertebrae). CT images were used to semi-automatically draw volumes of interest (VOI) on each AC site, using a region-growing algorithm whose lower limit was set at 130 HU. In each VOI, average HU was calculated. Thereafter, average SUV/counts were computed in each VOI using the co-registered PET data (from attenuation-correction and from non-attenuation-correction images, respectively). These values were normalized for blood-pool radioactivity, which was obtained by drawing a 10-slice thick VOI on the inferior vena cava, to obtain plaque target-to-background ratio (TBR).
During this phase, several focal areas of increased 18F-NaF uptake without apparent calcification at the coregistered CT images, were visually identified within the aortic wall. These foci were defined as “non calcified hot spots” (HS) and were selected for further analysis on the bases of criteria suggested by Tatsumi et al. for FDG (21): HS were analyzed if they presented an appreciable contrast with respect to the surrounding segments, on the basis of a blinded qualitative analysis performed by two experienced readers (SM and GMS). HS were thus segmented in a VOI using a 3D isocontour method, setting the lower threshold at 50% of the voxel with the highest SUV. This value was chosen as it allowed including the higher uptake area, limiting the confounding interference of partial volume effect and motion artifacts. Their average SUV was also normalized for blood-pool value to obtain HS TBR.

Finally, TBR was defined in a control region (CR), which was defined as a volume of interest, at least 5 slice thick, manually drawn in an arterial segment where neither calcium deposition nor increased 18F-NaF uptake was detected.

Image analysis was carried out with 64-bit Osirix DICOM viewer (Pixmeo, Geneva, CH) and with PMOD software package (v. 3.4, PMOD Technologies, Zurich, CH).

**Statistical Analysis**

All ACs were plotted and then stratified in tertiles, according to their average HU. Plaque whose mean HU was located in the lower tertile were defined Light Plaques (LP), while those having average HU in the intermediate and upper tertile were labeled as Medium and Heavy Plaques (MP and HP, respectively).

All data are reported as Mean±Standard Seviation. Differences between groups were tested using one-way analysis of variance, with intergroup comparison afforded using Bonferroni test. Two-
tailed Pearson R index was used to test the significance of correlations. Statistical analyses were performed using a dedicated software application (SPSS, v. 21.0)

RESULTS

Characteristics of CT and PET findings

A total of 397 ACs were identified in the entire population (6.2 AC per patient, on average). After stratification according to tertiles, HU values ranging from 130 to 210 defined LP, attenuation coefficient from 211 to 510 defined MP while HU>510 were considered HP. Mean attenuation coefficient of HS and of CR was respectively 46.2±11.3 and 41.6±9.9, without any significant difference between these two groups (p=ns). Every patient had at least one LP. MPs were relatively less common, as they could be found in 41 patients (64%). HPs were even less prevalent (31 patients, 48%). Non-calcified arterial HS were present in 55 patients (86% of total) accounting for a total of 189 areas (3.4 per patient, on average).

Arterial Plaque Uptake

Among plaques, LPs had the highest blood-pool corrected uptake: their average TBR was in fact 2.21±0.88. This value was significantly higher with respect to both MPs (1.59±0.63, p<0.01) and HPs (1.14±0.37, p<0.001). Similarly, MPs’ TBR was significantly greater than HPs’ (p<0.001, Figure 1).
Uptake of HPs was in effect so low to be undistinguishable from CR (1.16±0.52, p=0.87), while both MPs and LPs stood out when compared to these control segments (p<0.01 and p<0.001, respectively, Figure 1).

Overall, calcified plaque HU density showed a definite, inverse correlation with TBR when averaging, in each patient, all plaques of each class (R=0.7, P<0.01).

TBR obtained from non-attenuation-correction reconstruction algorithm was respectively 2.15±0.81, 1.6±0.45 and 1.05±0.39 for LPs, MPs and HPs. These values didn’t show any significant discordance from those obtained from the attenuation-weighted reconstruction method (Figure 2).

**Focal 18-F NaF Uptake Areas and Their Correlation with ACs**

Obviously, non-calcified arterial HS had an average TBR that was significantly increased with respect to any plaque type (3.89±1.87, p<0.0001, Figure 1). In each patient, average TBR of these HS strictly correlated with corresponding TBR value in LPs (R=0.8, p<0.01, Figure 3). The correlation was still detectable (albeit with less significance) when MPs were considered (R=0.42, p<0.01) while it disappeared when evaluating HPs (R=0.16, p=0.37).
DISCUSSION

In the present study, 18F-NaF evaluation of mineral metabolism within the arterial wall documented an inverse correlation between plaque calcium density and tracer avidity. Radioisotope accumulation within calcified plaque indeed peaked in lesions located at the lower end of HU coefficient, and progressively ebbed when progression towards heavier calcific concretions; accordingly, heavy plaques did not show substantial uptake differences with respect to control regions.

Interestingly, focal areas of 18F-NaF uptake could also be observed in calcium-free arterial regions in the vast majority of patients. These areas were not associated with significant increase in HU, confirming the divergent nature of mechanisms underlying visible calcium deposition and tracer uptake. However, the correlation between average TBR in these non-calcified HS and the corresponding mean values in light plaques suggests the presence of a connection between mechanisms governing radiotracer uptake within different regions of the vascular wall. Actually, present data do not allow establishing what do these focal uptake areas represent and whether these “hot spots” will subsequently evolve in a early-stage AC; further studies are presently needed, as to evaluate the evolutionary pattern of these findings at subsequent scans (18F-NaF PET/CT or also contrast-enhanced CT), as done by Adbeldaki et al. for inflammation detection with FDG uptake (22).

These characteristics of fluoride kinetics within vascular walls might account for the uneven pattern of $^{18}$F-Fluoride distribution within plaques. In fact, our data show that estimation of tracer uptake is strongly dependent on the plaque characteristics, as less CT-evident plaques, which would have scored low at the calcium score software assessment, are actually the ones that
concentrate the tracer more avidly. This implies that potentially reversible processes, active in fostering AC growth, are of relevance mainly on the earlier stages of plaque formation.

Conversely, denser and more CT-evident plaques, scoring high at calcium score determination, have a relatively lower fluoride uptake. Obviously, this doesn’t imply that the plaque won’t grow anymore (as gradient-based processes could cause further calcium salts precipitation), yet it suggests that this plaque stage is characterized by a relatively slower rate of inorganic calcium apposition. It can then be derived that radio-isotopic evaluation of arterial plaque is only feasible in these ACs that are still in the active phase of their existence, while greater and more dense calcification are to be considered “scar tissue” and should therefore excluded from any functional evaluation.

Our findings might thus explain the high variability that has been observed in the prevalence of “hot” plaques in different vascular districts, as plaques growing in vessels characterized by smaller diameter, such as the carotids and coronary arteries are more likely to be identified, even at an initial stage (23-25). Conversely, plaque development in greater vessel can be relatively asymptomatic; these arterial segments may thus house initial, intermediate and end-stage “cold” plaques without being clinically detected.

According to the present data, CT evidence of AC and PET evidence of 18F-NaF uptake might represent two different markers of atherosclerosis. The former seems to be an accurate index of total vascular damage describing the history of atherosclerosis progression and its duration throughout the arterial tree. The latter seems more closely related to the actual rate of plaque progression, mostly in its earlier stages.
This study presents some limitations. First, calcium-free HS areas were actually a rather common finding in our series. Although average attenuation coefficient of these vascular segments was not significantly different with respect to control areas, the limited spatial resolution of the “non-diagnostic” CT does not permit to exclude the presence of calcium micro-calcifications (26). Actually, this pattern has the ability of perpetuating the vicious circle of plaque inflammation, leading to continued plaque growth (27); a recent research by Joshi et al. demonstrated that plaques with increased 18F-NaF uptake present a greater prevalence of micro-calcifications, as well as greater expression of bone turnover markers (28). In this context, plaque assessment by 18F-NaF might thus represent a potentially useful tool in trials assessing the effectiveness of pharmacologic treatments to reduce atherosclerosis progression. Moreover, as previously reported (18), all patients enrolled in the analysis were affected by different oncological diseases with potential of bone marrow metastases, as this condition justifies the radiation burden associated to 18F-NaF PET/CT scan. Finally, even though pathological evidence of a link between plaques fluoride uptake and foci of osteoblast-like-cells activity is still limited, it is true that tracer uptake in the mineralization matrix has been validated in different experimental and clinical settings (29,30). The histopathological confirmation of the presence of shared mechanisms in bone-forming tissue and in the ongoing plaque (3) provides a solid ground, allowing the application of nuclear imaging method in the estimation of the plaque calcium metabolism.

**CONCLUSION**

Our data represent a possible interpretation of the functional 18F-NaF PET imaging of the arterial tree. In the presence of visible atherosclerotic damage, tracer uptake was inversely correlated with degree of calcium deposition. The different nature of information provided by CT and PET
suggests that the two techniques might represent two different windows on atherosclerosis progression, the former describing its past history and the latter its current progression rate. This integrated information might thus provide a more complete assessment of vascular damage and a more accurate evaluation of treatment effectiveness.

**DISCLOSURE**

None of the authors has any conflict of interest to disclose.
REFERENCES


Figure 1: PET/CT qualitative and semi-quantitative analysis of the three plaque subtypes, of the hot spots and of the control segments. On the upper panels it is shown the radiological aspect of the less dense plaques (LP), which show intense fluoride uptake. Denser types of plaques (MP) show less tracer accumulation, while plaques in the highest tertile of Hounsfield density show no visible uptake at all. The highest activity is actually recorded in the calcium-free arterial hot spots (HS). On the bottom left panel is represented the distribution of uptake intensity versus density of the plaque subtypes, of the hot spot and of control regions on a scatterplot graph, allowing to appreciate the distribution spread of each element and the inverse correlation between plaque density and TBR. On the bottom right panel, the histogram shows the significant difference between the different plaque subtypes and the substantial identity of fluoride uptake between heavy plaques and control region.
Figure 2: Estimated average uptake in the three plaque groups, reconstructed according to an attenuation-weighted algorithm and a non-attenuation correction protocol (upper panel). No substantial differences were noticed in any plaque type. Qualitative assessment (lower panels) didn’t show any artifactual uptake on AC reconstruction.
Figure 3: Patient-by-patient correlation between the average of all HS and LPs (top), MPs (middle) and HPs (bottom). The significance of this correlation markedly dwindle when progressing from LPs to HPs, suggesting that lighter plaques share, at least in part, the mechanisms active within hot-spots.
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