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CRedit author statement

Kamran Majeed: validation, formal analysis, investigation, OCT analysis, writing original draft; **Jamie Bellinge:** formal analysis, validation, investigation, PET image analysis and interpretation; **Steele Butcher:** validation, investigation, CTCA analysis and interpretation; **Richard Alcock:** investigation, writing review and editing; **Jon Spiro:** investigation, writing review and editing; **David Playford:** writing - review & editing; **Graham Hillis:** writing - review & editing; **David Newby:** writing - review & editing; **Roslyn Francis:** conceptualisation, methodology, supervision, funding acquisition; **Carl Schultz:** conceptualisation, methodology, supervision, funding acquisition.

Coronary ^{18}F -sodium fluoride PET detects high-risk plaque features on optical coherence tomography and CT-angiography in patients with acute coronary syndrome

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ABSTRACT

Background and aims

^{18}F -Sodium Fluoride Positron Emission Tomography (^{18}F -NaF PET) non-invasively detects micro-calcification activity, the earliest stage of atherosclerotic arterial calcification. We studied the association between coronary ^{18}F -NaF uptake and high-risk plaque features on intra-coronary optical coherence tomography (OCT) and CT-angiography (CTCA) and the potential application to patient-level risk stratification.

Methods:

Sixty-two prospectively recruited patients with acute coronary syndrome (ACS) underwent multi-vessel OCT, ^{18}F -NaF PET and CTCA. The maximum tissue to background ratio (TBRmax= standardised uptake value (SUV)max/SUVbloodpool) was measured in each coronary segment on ^{18}F -NaF PET scans. High-risk plaque features on OCT and CTCA were compared in matched coronary segments. The number of patients testing positive ($>2\text{SD}$ above the normal range) for micro-calcification activity was determined.

Results:

In 62 patients (age, mean $\pm$ 95% confidence interval (CI) = 61 $\pm$ 9 years, 85% male) the coronary segments with elevated ^{18}F -NaF uptake had higher lipid arc (LA) (median [25th-75th centile]: 74° [35°-117°] *versus* 48° [15°-83°], $p=0.021$), higher prevalence of macrophages [n(%): 37 (62%) *versus* 89 (39%), $p=0.008$] and lower plaque free wall (PFW) (50° [7°-110°] *versus* 94° [34°-180°], $p=0.027$) on OCT, and a higher total plaque burden ($p=0.011$) and higher dense calcified plaque burden ($p=0.001$) on CTCA, when compared with ^{18}F -NaF negative segments.

Patients grouped by increasing number of coronary lesions positive for microcalcification activity (0,1, ≥ 2) showed decreasing plaque free wall, increasing calcification and increasing macrophages on OCT (respectively $p=0.008$, $p<0.001$ and $p=0.028$).

Conclusions:

^{18}F -NaF uptake is associated with high-risk plaque features on OCT and CTCA in a per-segment and per-patient analysis in subjects hospitalized for ACS.

Keywords:

^{18}F Sodium Fluoride Positron Emission Tomography, Microcalcification, Acute coronary syndrome, Optical coherence tomography,

1 Introduction:

A significant proportion of patients with acute coronary syndrome (ACS) suffer from recurrent major adverse cardiovascular events following their index presentation, despite recent advances in pharmacological and invasive therapies (1). The majority of the atherosclerotic lesions responsible for recurrent events are non-flow limiting and cannot be identified on routine coronary angiography (2). Histological studies of ACS non-survivors have identified a high-risk phenotype of coronary plaque rich in lipid, inflammatory cells and covered by a thin fibrous cap (2), raising the prospect that *in-vivo* plaque characterisation and plaque relevant biomarkers may refine risk stratification of high-risk patients. Studies using invasive intravascular imaging have demonstrated that *in-vivo* plaque morphology provides prognostic information and may improve patient risk stratification (1).

Recent studies demonstrate the feasibility and reproducibility of non-invasive coronary imaging with ^{18}F -sodium fluoride positron emission tomography (^{18}F -NaF PET), which enables novel insights into the pathophysiology of atherosclerosis and vascular calcification (3,4). ^{18}F -NaF incorporates into hydroxyapatite crystals and detects micro-calcification activity, the earliest and most active stage of arterial calcification that translates within a short period of time into new computed tomography (CT)-detectable macrocalcification (5). In addition, ^{18}F -NaF PET is able to identify culprit plaques in ACS patients (6). However, there are limited data on whether ^{18}F -NaF tracer uptake is associated with high-risk plaque features in ACS.

Optical coherence tomography (OCT) is a high-resolution intravascular imaging modality with the ability to characterize plaque features that would otherwise only be detectable by histology, including those implicated in plaque progression and destabilization such as thin fibrous caps, macrophages, microchannels and cholesterol crystals (7). We aimed to

investigate whether the high-risk plaque features identified by OCT are associated with uptake of ^{18}F -NaF in a per-coronary segment and per-patient analysis among prospectively recruited patients with ACS. We also evaluated plaque features on CT-coronary angiography (CTCA).

2 Patients and methods

2.1 Patient recruitment

ACS patients undergoing in-hospital coronary angiography were prospectively recruited from the Royal Perth Hospital (RPH) between November 2015 and February 2018 as part of the multi-modality imaging and biomarkers to improve risk-stratification for secondary prevention after acute coronary syndrome (MOTIVATOR) study. The study was registered with the Australian New Zealand Clinical Trial Registry (<http://www.anzctr.org.au:ACTRN12615001234505>). An additional ten patients without prior cardiovascular events and negative coronary calcium scores were recruited and underwent ^{18}F -NaF PET to define the upper limit of the normal reference range.

Exclusion criteria were: 1) glomerular filtration (GFR) <40 ml/min or <60 ml/min with diabetes mellitus, 2) unlikely to survive for at least three years due to existing medical comorbidities, 3) coronary anatomy unsuitable for OCT, 4) pregnancy, 5) prior coronary artery bypass grafting, or 6) ST-segment elevation myocardial infarction treated with a primary percutaneous coronary intervention. The study was approved by the Royal Perth Hospital Human Research Ethics Committee (MOTIVATOR Ref: REG 2015-033). All participants provided written informed consent before in-hospital coronary angiography.

2.2 OCT image acquisition and analysis

OCT imaging was performed at the time of coronary angiography. Two or three epicardial coronary arteries were selected on the basis that OCT imaging could be safely performed in the proximal 50 mm of each vessel either pre- or post-stenting at the discretion of the interventional cardiologist.

All OCT images were anonymized and analyzed using proprietary software on a dedicated workstation (OPTIS Offline Reviewer, St. Jude Medical, St. Paul, MN, USA). Plaque features including lipid arc (LA), calcium arc (CA) and plaque free wall (PFW), macrophages, microchannels, cholesterol crystals, thrombus and thin cap fibroatheroma were analyzed using previously published methods of OCT plaque analysis(7-9). A detailed description of OCT image acquisition and analysis is provided in the Supplementary Data.

2.3 ^{18}F -NaF PET/CT image acquisition and analysis

Participants underwent ^{18}F -NaF PET as soon as possible and within a maximum time window of up to 4 weeks of hospital discharge. Participants were injected with 250 MBq of ^{18}F -NaF and rested for 60 minutes prior to imaging. An attenuation correction CT-scan was performed first (120 kV, 50 mAs), followed by 10 minutes of free breathing electrocardiograph gated (ECG) list-mode PET acquisition using a Siemens Biograph mCT 64 PET/CT scanner. An ECG-gated, end-expiratory CT-coronary angiography (CTCA) was performed within two weeks of the ^{18}F -NaF PET scan, using either a Phillips iCT with IMR or Siemens Definition AS+. Where needed, subjects were pre-treated with oral beta-blocker therapy to obtain a target heart rate <65 beats per minute, before PET and CTCA imaging.

The diastolic PET images, representing 50-75% of the cardiac cycle, were fused with the CTCA images, using a previously established method (10). Fused PET/CTCA scans were

analyzed by experienced observers, blinded to OCT data, using Siemens Syngo.via VB10B. Coronary artery ^{18}F -NaF PET analysis was performed by drawing 2D freehand regions of interest (ROI) around the adventitial border of major coronary arteries (>2 mm in diameter) on 3-mm axial slices (6). Maximum radiotracer activity was recorded from each ROI and adjusted for blood pool activity by drawing a large spherical ROI in the right atrium, resulting in the maximum tissue-to-background ratio ($\text{TBR}_{\text{max}} = \text{standardised uptake value (SUV)}_{\text{max}} / \text{SUV}_{\text{bloodpool}}$) (11).

A low-risk cohort ($n=10$) with no prior coronary heart disease and coronary calcium scores equal to zero were used to determine an upper limit of the normal reference range of radiotracer activity for each coronary segment based on the 18-segment model. For each coronary segment, the maximum ^{18}F -NaF activity was measured and thereafter the mean ± 2 standard deviations (SD) was calculated for the group (Supplementary Table S1). Thereafter, micro-calcification activity was deemed to be present in the ACS cohort in coronary segments with radiotracer activity $>2\text{SD}$ above the mean obtained in the low-risk cohort. Coronary segments with TBR_{max} values determined to be positive, but with PET activity appearing to originate from an adjacent non-coronary structure (eg. aortic valve or annulus) were deemed ^{18}F -NaF overspill and were considered as ^{18}F -NaF negative.

2.4 Quantification of plaque burden on CTCA

Semi-automatic quantification of coronary plaque burden was performed utilizing previously validated, dedicated CTCA software (QAngio CT RE, Version 3.1, Medis, Leiden, the Netherlands) by a single reader who was blinded to clinical data and all other imaging data (12,13). An automatic tree extraction algorithm was used to derive initial straightened and curved multi-planar reformatted (MPR) volumes for each coronary vessel. Luminal and vessel wall contours were then analysed in 0.5 mm transverse cross-sections perpendicular to derived centrelines. Classification into low attenuation, fibro-fatty, fibrotic and dense

calcified plaque was automated based on dynamic adaptive Hounsfield Unit (HU) thresholds adjusted according to an algorithm based on luminal contrast densities, a method previously validated by intravascular ultrasound (IVUS)(13). Plaque quantification was performed in all coronary vessels until luminal diameter was less than 2 mm. Plaque burden was calculated as $(\text{plaque area} / \text{vessel area}) \times 100$. The stented sections of each segment were excluded from the final analysis.

2.5 Co-registration and labelling of all imaging according to the 18-segment model

The steps were 1) to harmonize the labelling of coronary segments on coronary angiography and CTCA; 2) co-registration of CTCA with PET-CT and 3) labelling of coronary segments on OCT by comparison with coronary angiography. More information on each step is provided in the Supplementary Data. Co-registration was performed by two observers, blinded to both OCT and ^{18}F -NaF PET findings. PET and OCT images were analyzed separately and independently each by a single different observer, blinded to the other complementary (PET or OCT) imaging data, and geographically separated from the other observer. We previously demonstrated high inter-observer reproducibility using these techniques for ^{18}F -NaF PET (11). For OCT, intra-observer variability was assessed by repeating the plaque analysis three months after the initial analysis. Inter-observer variability was examined by repeating the plaque analysis on randomly selected OCT frames amongst 2 different OCT observers blinded and geographically separated.

2.6 Statistical analysis

Categorical variables are presented as counts: n (%). Continuous variables are expressed as the mean $\pm$ SD or as median [25th, 75th centile] dependent on whether the variable distribution was Gaussian.

For segment level OCT analysis, the mean values of LA, CA and PFW were calculated for PET/OCT co-registered coronary segments. The presence of macrophages, microchannels and cholesterol crystals was also recorded in individual coronary segments imaged. To investigate segment level associations between elevated ^{18}F -NaF uptake and high-risk OCT plaque features, a generalized estimating equation (GEE) model was used to adjust for intra-individual effects. Due to the collinearity amongst various plaque features, the analyses were performed in two separate models. Model 1 included LA, CA, microchannels and cholesterol crystals. Model two included PFW, macrophages, presence of thin cap fibroatheroma and presence of thrombus. A univariate analysis was performed to compare the TBRmax values between ^{18}F -NaF positive and negative coronary segments. The association between various CTCA plaque features and ^{18}F -NaF was studied using a GEE model after adjusting for intra-individual effects.

For patient-level analysis, the mean values of LA, CA and PFW were calculated from all multi-vessel OCT pullbacks for each patient. Positivity for lipid, calcium and PFW was defined as being above the median for the whole group. Patients were deemed positive for macrophages when these were present in more than 50% of coronary segments. Patients were considered positive for cholesterol crystals and micro-channels if these micro-features were detected in one or more of the coronary segments.

Independent predictors of positive ^{18}F -NaF uptake in individual patients with ^{18}F -NaF uptake *versus* those without ^{18}F -NaF uptake were analyzed using a binary logistic regression model. Individual clinical and plaque features with significant predictive value ($p < 0.1$) on univariate analysis were further evaluated with multivariate logistic analysis. Backward elimination was used to determine the most parsimonious multivariate model. Patients were categorized into three groups according to the number of coronary segments with elevated micro-calcification

activity (0, 1 or 2 or more). The Chi-square test for trend was used to evaluate the difference in various plaque features across the three groups of patients(14).

Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software version 27.0 (IBM Corp., Armonk, NY, USA). A two-sided $p < 0.05$ was considered significant.

3 Results:

A total of 62 patients (mean age 61 ± 9 years, 85% male) were included, and OCT imaging was obtained in 286 coronary segments (Figure 1). The demographic, procedural and imaging characteristics of the patients are shown in Table 1.

3.1 Segment level analysis

Of the 286 coronary segments with ^{18}F -NaF PET and OCT data, a total of 60 (21%) showed elevated ^{18}F -NaF uptake (Table 2). In Model 1, coronary segments with elevated (positive) ^{18}F -NaF uptake compared to negative uptake showed significantly higher LA (74° [35° - 117°] *versus* 48° [15° - 83°]; beta coefficient (β)=0.06, 95% confidence interval (CI) (0.01-0.12), $p=0.021$), CA (8° [0° - 40°] *versus* 0° [0° - 6°]; β =0.19 (95% CI 0.07-0.31), $p=0.003$), and cholesterol crystals [11 (18.3%) *versus* 14 (6.2%); β =1.144 (95% CI 0.28- 2.006), $p=0.009$] (Table 2). In Model 2, PFW was significantly lower in ^{18}F -NaF positive segments (50° [7° - 110°] *versus* 94° [34° - 180°]; β =-0.05 (95% CI -0.09- -0.01), $p=0.027$) and the prevalence of macrophages was higher [37 (62%) *versus* 89 (39%); β =0.766 (95% CI 0.203- 1.33), $p=0.008$].

In a sensitivity analysis, coronary segments deemed ^{18}F -NaF positive due to overspill from adjacent structures showed plaque features in-between those of definite ^{18}F -NaF positive and negative segments (Supplementary Table S2).

Of 661 coronary segments with both CTCA and ^{18}F -NaF PET data, there were 79 with elevated ^{18}F -NaF uptake and 582 that were negative for ^{18}F -NaF uptake. Coronary segments with ^{18}F -NaF uptake demonstrated higher total plaque burden ($\beta = 0.004$ [0.001 to 0.008], $p = 0.011$) and higher dense calcified plaque burden ($\beta = 0.041$ [0.017 to 0.065], $p = 0.001$) in univariate analysis (Supplementary Table S3). When both CTCA total plaque and dense calcified plaque burden were included in the same model, only dense calcified plaque burden remained significant ($p = 0.01$).

3.2 Patient level analysis

In the total patient cohort, the distributions of plaque features on OCT were as follows: LA 59° [39° - 80°], CA 6° [1° - 15°] and PFW 102° [68° - 146°], while the plaque micro-features were detected as follows: macrophages 13 (21%), micro-channels 31 (50%), cholesterol crystals 22 (35%) and thin cap fibroatheroma 19 (31%).

Twenty patients had no ^{18}F -NaF positive lesion and 42 patients had one or more ^{18}F -NaF positive lesions. PET positivity was associated with increased calcium and decreased plaque free wall on OCT, but not with any plaque features on CTCA. In a multivariate analysis, OCT calcium (odds ratio= 15.9 [2.2- 115.6], $p = 0.006$) and PFW (odds ratio= 0.1 [0.01-0.6], $p = 0.013$), age (odds ratio=0.8 [0.7-0.9], $p = 0.004$) and prescription of calcium channel blockers (odds ratio= 0.06 [0.00- 0.84], $p = 0.037$) were independently associated with ^{18}F -NaF uptake.

Twenty patients had no ^{18}F -NaF positive lesion, 21 patients had one ^{18}F -NaF positive lesion, and 21 patients had two or more ^{18}F -NaF positive lesions. The proportion of patients with calcium arc above the group median increased across the three groups, with an increasing

number of ^{18}F -NaF positive lesions, whereas PFW above the group median decreased across the three groups (Figure 2 and Supplementary Table S4). The proportion of patients with OCT detected macrophages also increased across the three groups (Figure 2 and Supplementary Table S4). There were no associations with CTCA plaque features across the 3 patient groups.

3.3 Reproducibility of OCT measurements

Intra-observer variability was assessed on 200 (100 pre-stenting, 100 post-stenting) frames on randomly selected patients. There was excellent test-retest reproducibility for LA (intra-class correlation, $R=0.96$, 95%CI 0.95-0.97), CA ($R=0.98$, 95%CI 0.97-0.98), macrophages ($R=0.91$, 95%CI 0.88-0.93), microchannels ($R=0.85$, 95%CI 0.81-0.89) and cholesterol crystals ($R=0.95$, 95%CI 0.93-0.96).

Inter-observer agreement assessed on 150 (80 pre-stenting and 70 post-stenting) OCT frames yielded excellent concordance for LA ($R=0.97$, 95% CI 0.96-0.98), CA ($R=0.95$, 95% CI 0.94-0.97), macrophages ($R=0.87$, 95% CI 0.82-0.90) and cholesterol crystals ($R=0.86$, 95% CI 0.81-0.89). There were no frames with microchannels.

Discussion

To the best of our knowledge, this is the only study to date investigating the association between ^{18}F -NaF PET uptake and high-risk atherosclerotic plaque detected by intracoronary OCT in patients presenting with ACS. The coronary segments with elevated ^{18}F -NaF had more LA, CA, macrophages and cholesterol crystals, and less PFW. Furthermore, on a patient level, the detection of coronary ^{18}F -NaF PET-positive lesions identified patients with high-risk plaque features.

Our data demonstrated that ^{18}F -NaF uptake was associated with higher plaque calcium, macrophages and cholesterol crystals and lower PFW. Dweck et al. reported that ^{18}F -NaF activity was strongly associated with CT-calcium score in stable patients (3). Joshi et al. reported that in 40 patients with stable angina coronary lesions, ^{18}F -NaF uptake was associated with more high-risk features on intravascular ultrasound including higher remodelling index, microcalcification and necrotic core (6). PFW is validated as an OCT surrogate for plaque burden and represents the absence of vessel wall remodelling due to early atherosclerosis (9). In our study, PFW was inversely associated with ^{18}F -NaF uptake at segmental as well as a patient level. These data suggest that the absence of coronary ^{18}F -NaF uptake may identify a subgroup of patients with ACS with an earlier stage of atherosclerosis likely to be at relatively low risk of future recurrent events.

We report an association of ^{18}F -NaF uptake with plaque macrophages and cholesterol crystals. These findings are consistent with the concept that ^{18}F -NaF binds to the areas of early and active calcification, likely triggered by plaque inflammation, and intriguingly suggests a potential role for the nucleotide-binding domain like receptor protein-3 inflammasome in early calcification (15). Lee et al. (16) studied 52 stable patients, excluding ACS, with OCT, intra-vascular ultrasound (IVUS) and CTCA, and reported that ^{18}F -NaF uptake was associated with plaque burden and remodelling index on IVUS and higher lipid arc on OCT, but not with other plaque features including calcium or macrophages. In contrast, our study involved patients with ACS and used a higher sampling rate / smaller sampling interval for analyzing OCT plaque micro-features, as we have previously shown this to be optimal to avoid underestimation of plaque micro-features including macrophages, micro-channels and cholesterol crystals (8). In contrast to these previous studies (6,16), we

also conducted a patient-level analysis based on the number of ^{18}F -NaF positive lesions, which may be helpful in clinical translation.

We found that ^{18}F -NaF uptake was associated with total plaque burden and dense calcified plaque burden but not with low attenuation plaque on CTCA. Other studies have shown that total plaque burden is consistently one of the best CVD risk predictors irrespective of which imaging modality is used to quantify it. Similar observations apply to coronary calcification burden, which is a good surrogate for total plaque burden. The association of total and calcified plaque on CTCA with ^{18}F -NaF uptake is consistent with similar associations with PFW and calcium on OCT observed in our study. In contrast, we observed an association of lipid and macrophages on OCT, but not with low attenuation plaque on CTCA, which might be anticipated to be co-located. OCT has a considerably higher resolution than CTCA and a high reproducibility for the measurement of lipid and macrophages. In our experience, the quantification of low attenuation plaque burden on CTCA is somewhat less reproducible than that of other plaque types, even using our semi-automated and elsewhere validated CTCA plaque analysis methods. Furthermore, the plaques with the lowest attenuation were most likely already stented and therefore excluded from our CTCA analysis. Given these factors, our study probably did not have sufficient statistical power to detect a significant difference in low attenuation plaque burden on CTCA between ^{18}F -NaF positive and negative segments. Further studies are required to specifically address this question.

In our study, 42 out of 62 (68%) subjects with ACS demonstrated increased coronary ^{18}F -NaF uptake. Joshi et al. (6) reported, in a study of 40 subjects who had ACS, that 93% were ^{18}F -NaF positive. While the TBRmax values reported in their study (6) were similar to our findings, other important differences may in part explain the lower prevalence of PET-

positive lesions described in our study. We used a higher threshold for ^{18}F -NaF positivity, based on the upper limit of the normal range for our methods. Furthermore, the degree of calcification in culprit lesions in our patients was heterogeneous. Importantly, we used a higher dose of 250 MBq of ^{18}F -NaF with a 1-hour uptake phase and a shorter image acquisition time of 10 minutes compared to 125 MBq dose with a 1-hour uptake phase and 20-30 minute acquisition time (6). The shorter acquisition time reduces the likelihood of image degradation due to patient movement/discomfort but requires a higher radiotracer dose to retain sufficient counts and to limit false-positive findings due to 'noise' in the images. The higher threshold for lesion positivity in our study, based on our normal range, should reduce false positivity with the trade-off of missing some mildly positive lesions. The feasibility and reliability of ^{18}F -NaF to identify ruptured and high risk atherosclerotic plaques has been reported previously (4). Additionally, different approaches to vascular ^{18}F -NaF PET analysis have been proposed (17) and further ongoing studies will clarify the role of each and lead to technological improvements in coronary PET imaging.

We found that patients with ACS with an increasing number of ^{18}F -NaF positive coronary lesions showed an increasing prevalence of high-risk plaque features. Prior studies that investigated the ^{18}F -NaF uptake and high-risk plaque features on intra-coronary imaging included only patients with stable coronary artery disease and may therefore not be directly comparable, as there are recognized differences in plaque morphology between ACS and stable patients. Our results support the concept that micro-calcification activity may identify a subset of patients with ACS who are at high risk of further events that may benefit from more aggressive risk factor modification or plaque stabilization therapies. Interestingly, a recent study combining cohorts from two centres reported that increased coronary ^{18}F -NaF PET uptake was a better predictor of CVD events over the subsequent 4 years than risk scores or

clinical factors in a population of predominantly (79%) stable patients (18). Future prospective studies are needed to assess the clinical utility of ^{18}F -NaF PET imaging to predict major adverse cardiovascular outcomes after ACS.

4.1 Limitations

Our study is the largest cohort of ACS patients undergoing assessment using ^{18}F -NaF and intracoronary OCT to date but nonetheless represents only a modest sample size. On average, 5 coronary segments were imaged with OCT per patient (286 segments/62 patients) in at least 2 of the 3 major epicardial arteries, of which the proximal 50 mm of each coronary artery was always imaged. It is recognized that the vast majority of plaque events occur within the proximal 50 mm from the ostium (19). It follows that the under-sampling of the distal vessels and smaller side branches would have a limited effect on the determination of a patient's atherosclerosis phenotype. These smaller segments often cannot be imaged with OCT for technical reasons. While the interpretation of OCT appearances representing various plaque features is broadly accepted (7), the interpretation of bright spots representing macrophages has been challenged. At our institution, 75% of patients undergoing in-patient coronary angiography following ACS are male, and this is reflected in the demographic of our study population. As a result of the small number of females included in our study, the study findings may not be generalizable to the female sex. The cut-offs to define abnormal plaque features on OCT were based on the statistical distribution in our population and require further validation.

4.2 Conclusion

Our study demonstrates that coronary artery ^{18}F -NaF uptake is associated with multiple high-risk OCT plaque features and has the potential to refine the risk stratification of patients with ACS.

Declaration of competing interests

CJS received a research grant and honoraria unrelated to this research from Abbott Vascular Ltd. The other authors have nothing to disclose.

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CRediT author statement

Kamran Majeed: validation, formal analysis, investigation, OCT analysis, writing original draft; **Jamie Bellinge:** formal analysis, validation, investigation, PET image analysis and interpretation; **Steele Butcher:** validation, investigation, CTCA analysis and interpretation; **Richard Alcock:** investigation, writing review and editing; **Jon Spiro:** investigation, writing review and editing; **David Playford:** writing - review & editing; **Graham Hillis:** writing - review & editing; **David Newby:** writing - review & editing; **Roslyn Francis:** conceptualisation, methodology, supervision, funding acquisition; **Carl Schultz:** conceptualisation, methodology, supervision, funding acquisition.

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Figure 1: Flowchart showing the recruitment of patients in this study

Table 1: Baseline demographic, clinical and imaging characteristics.

	All subjects (n=62)	¹⁸ F-NaF +ve subjects (n=42)	¹⁸ F-NaF -ve subjects (n=20)	p value
Characteristics on admission				
Gender (males%)	53 (86%)	41 (97.6%)	12 (60%)	0.003
Age (years)	61 (9)	59 (9)	65 (7)	0.025
Height (cm)	175 (8)	176 (7)	172 (8)	0.032
Weight (kg)	90 (22)	93 (23)	82 (17)	0.063
Systolic blood pressure (mmHg)	132 (22)	132 (23)	134 (21)	0.632
Diastolic blood pressure (mmHg)	77 (15)	75 (16)	79 (14)	0.378
Heart rate (beats per minute)	72 (12)	70[62-79]	72 [62-84]	0.895
Known coronary / peripheral circulatory disease	5 (8)	3 (7)	2 (10)	0.701
Dyslipidaemia	28 (45)	18 (43)	10 (50)	0.598
Smoking	19 (30)	12 (29)	7 (35)	0.608
Diabetes mellitus	11 (18)	7 (17)	4 (20)	0.748
Anti-hypertensive therapy	34 (55)	20 (48)	14 (70)	0.103
Haemoglobin (g/dL)	146 [135-154]	146 [135-152]	142 [133-156]	0.768
Haematocrit	0.43 [0.40-0.45]	0.43 [0.40-0.44]	0.44[0.39-0.46]	0.783
White blood cells x 10 ⁹ /l	9.8 [8.3-11.9]	9.7 [8.3-10.9]	11 [8-12]	0.302
Creatinine (μmol/l)	77 [72-93]	73 [65-82]	78 [74-93]	0.725
Low density lipoprotein (mmol/l)	3.1 [2.5-3.5]	3.15 [2.67-3.62]	2.85 [2.2-3.47]	0.232
High density lipoprotein (mmol/l)	1.0 [0.9-1.25]	1.0 [0.85-1.20]	1.05 [0.9-1.30]	0.087
High sensitivity Troponin (ng/dL)	1185 [163-9607]	1485 [305- 13150]	698 [113-4418]	0.305
CRP (μg/mL)	5.2 [2.8-13.5]	5.4 [2.65-11.65]	5.0 [2.65-22.85]	0.999
HbA1c (%)	5.6 [5.5-6.0]	5.7 [5.5-6.0]	5.6 [5.4-6.1]	0.521
Albumin (g/l)	41 [39-44]	40.5 [39-44]	41 [38.75-44]	0.764
Medications on discharge				
ACE inhibitor	32 (52)	23 (52)	10 (50)	0.861
Angiotensin receptor blocker	11 (18)	8 (19)	3 (15)	0.697
Calcium channel blocker	7 (11)	2 (4.8)	5 (25)	0.033
Diuretics	6 (10)	3 (7)	3 (15)	0.338
Beta blockers	50 (81)	34 (81)	16 (80)	0.929
Statin	58 (94)	40 (95)	18 (90)	0.235
Antiplatelet	58 (94)	41 (98)	19 (95)	0.149
OCT procedural data				
Fluoroscopy time (mins)	16.1 [12.4- 24.9]	15.3 [11.2- 25.2]	15.3 [11.2- 25.2]	0.342
Total contrast (ml)	231.5 [195-276]	230 [183- 328]	230 [183- 328]	0.799
Vessels imaged per patient	2.1 (0.4)	2.0 (0.5)	2.1 (0.3)	0.505
Total length imaged/patient (mm)	104 [90-128]	97 [86-122]	97 [86 -122]	0.574
Frequency of vessels imaged				
Left main stem	32 (52)	23 (55)	9 (45)	0.473

Left anterior descending artery	59 (95)	41 (98)	18 (90)	0.288
Left circumflex artery	40 (65)	23 (55)	17 (85)	0.027
Right coronary artery	28 (45)	21 (50)	7 (35)	0.27
¹⁸F-NaF procedural data				
Time between OCT and PET imaging (days)	10 [6-11]	9 [4-11]	11 [7-20]	0.098
¹⁸ F-NaF dose (MBq)	249 [248-250]	249[248-250]	249 [248- 251]	0.204
Time between injection and PET imaging (min)	63 [60-68]	62 [60-67]	65 [59-74]	0.423
OCT plaque features				
Presence of lipid	31 (50)	23 (55)	8 (40)	0.28
Presence of calcium	31 (50)	27 (64)	4 (20)	0.002
Presence of plaque free wall	31 (50)	16 (38)	15 (75)	0.009
Presence of macrophages	13 (21)	11 (26)	2 (10)	0.159
Presence of thin cap fibroatheroma	19 (31)	14 (33)	5 (25)	0.507
Presence of thrombus	5 (8)	3 (7)	2 (10)	0.701
CTCA plaque features				
Total plaque burden (%)	38.3 [35.0- 43.3]	39.2 [35.5- 44.0]	38.3 [34.9- 41.6]	0.509
Low attenuation plaque burden (%)	3.4 [2.8-4.1]	3.3 [2.8- 4.1]	3.5 [3.1 -3.9]	0.559
Dense calcified plaque burden (%)	1.15 [0.48- 3.3]	1.79 [0.48- 3.93]	0.81 [0.49 -1.71]	0.1
Fibrous plaque burden (%)	22.8 [20.6-25.3]	22.6 [20.6-25.2]	22.9 [21.5- 25.0]	0.747
Fibrofatty plaque burden (%)	9.8 [8.5-10.6]	9.8 [8.7- 10.8]	10.0 [8.2- 11.0]	0.963
Non-calcified plaque burden (%)	35.7 [33.1-39.0]	35.5 [32.7- 39.2]	36.4 [33.8- 38.3]	0.58

Data is represented as n (%), mean $\pm$ standard deviation or median [25th-75th percentile]

Table 2: Comparison of anatomical distribution and plaque features between coronary segments with and without elevated ^{18}F -NaF uptake.

	^{18}F -NaF negative	^{18}F -NaF positive	β (95% CI)	<i>p</i> -value
Segments analysed (n)	226	60		
Max TBR	0.9 [0.76-1.05]	1.33 [1.18-1.56] ^a		
<u>Coronary distribution</u>				
Left anterior descending artery	109 (48)	37 (63)		
Left circumflex artery	45 (20)	17 (28)		
Right coronary artery	72 (32)	6 (10)		
OCT plaque features				
			Model 1^b	
Lipid arc (degrees)	48 [15-83]	74 [35-117]	0.06 (0.01-0.12)	0.021
Calcium arc (degrees)	0 [0-6]	8 [0-40]	0.19 (0.07-0.31)	0.003
Micro-channels	34 (15)	8 (13)	0.66 (-0.31-1.63)	0.185
Cholesterol crystals	14 (6.2)	11 (18)	1.14 (0.28-2.0)	0.009
			Model 2^b	
Plaque free wall (degrees)	94 [34-180]	50 [7-110]	-0.05 (-0.09- -0.01)	0.027
Macrophages	89 (39)	37 (62)	0.77 (0.20-1.33)	0.008
Thrombus	3(1.3)	2 (3.4)	0.72 (0.90-2.33)	0.384
Thin cap fibro-atheroma	24 (10)	10 (17)	0.07 (0.94-1.1)	0.896

^a *p*-value ≤ 0.001 in univariate analysis.^b Co-linearity between plaque variables necessitated the use of separate models

Data is represented as n (%) or median [25th-75th percentile]

Figure 2: Prevalence of selected plaque features above the group median, as detected on optical coherence tomography (OCT) across three groups of patients, with the increasing number of coronary lesions positive for micro-calcification activity. *p*-values represent the Chi-square test for trend.

Journal Pre-proof

Coronary 18F-Sodium Fluoride PET detects High-Risk Plaque features on Optical Coherence Tomography and CT-angiography in patients with Acute Coronary Syndrome

Highlights

- Coronary 18F-sodium fluoride uptake identifies plaque with higher lipid, macrophages and cholesterol crystals and with lower plaque free wall on optical coherence tomography.
- Coronary 18F-sodium fluoride uptake identifies plaque with higher total plaque burden and dense calcified plaque burden on CT-coronary angiography.
- Patients with an increasing number of 18F-fluoride positive coronary lesions have a plaque phenotype with higher calcification and macrophages and lower plaque free wall.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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