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Coronary 18F-Sodium Fluoride Uptake Predicts Outcomes in Patients With Coronary Artery Disease

[18]F-Sodium Fluoride Coronary Uptake Predicts Outcome in Patients with Coronary Artery Disease

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Brief Title: ^{18}F-NaF uptake predicts outcome

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Tweet: ^{18}F-NaF uptake is a powerful marker of active disease and predicts future myocardial infarction in patients with established coronary events.

ABSTRACT

Background: We lack reliable methods for predicting myocardial infarction in patients with established coronary artery disease. Coronary ^{18}F-sodium fluoride (^{18}F-NaF) positron emission tomography (PET) provides an assessment of atherosclerosis activity.

Objectives: We assessed whether ^{18}F-NaF PET predicts myocardial infarction and provides additional prognostic information to current methods of risk stratification.

Methods: Patients with known coronary artery disease underwent ^{18}F-NaF PET computed tomography and were followed-up for fatal or non-fatal myocardial infarction over 42 [31-49] months. Total coronary ^{18}F-NaF uptake was determined using coronary microcalcification activity (CMA).

Results: In a post-hoc analysis of data collected for prospective observational studies we studied 293 study participants (65±9 years; 84% male), of whom 203 (69%) showed increased coronary ^{18}F-NaF activity (CMA>0). Fatal or non-fatal myocardial infarction occurred only in patients with increased coronary ^{18}F-NaF activity (20/203 CMA>0 versus 0/90 CMA=0; p<0.001). On receiver operator-curve analysis, fatal or non-fatal myocardial infarction prediction was highest for ^{18}F-NaF CMA, outperforming coronary calcium scoring, modified Duke coronary artery disease index, REACH and SMART risk scores (areas under curve: 0.76 versus 0.54, 0.62, 0.52 and 0.54; p<0.001 for all). Patients with CMA>1.56 had >7-fold increase in fatal or non-fatal myocardial infarction (hazard ratio 7.1, 95% confidence interval 2.2 to 25.1; p=0.003) independent of age, gender, risk factors, segment involvement and coronary calcium scores, presence of coronary stents, coronary stenosis, REACH and SMART scores, the Duke coronary artery disease index and recent myocardial infarction.

Conclusion: In patients with established coronary artery disease, ^{18}F-NaF PET provides powerful independent prediction of fatal or non-fatal myocardial infarction.

Keywords: ^{18}F-NaF PET, coronary computed tomography, coronary artery disease, myocardial infarction, coronary event risk prediction

Condensed abstract

We assessed whether ^{18}F-NaF PET predicts myocardial infarction and provides additional prognostic information to current methods of risk stratification. Patients with known coronary artery disease underwent contrast-enhanced ^{18}F-NaF PET computed tomography and were followed-up for myocardial infarction over 42 [31-49] months. Among 293 study participants myocardial infarction occurred only in patients with increased coronary ^{18}F-NaF activity. Patients with increased ^{18}F-NaF uptake had >7-fold increase in myocardial infarction independent of age, gender, cardiovascular risk factors, segment involvement scores, presence of coronary stents, number of vessels with significant stenosis, coronary calcium scoring, REACH and SMART scores, the Duke index, initial patients presentation (acute coronary syndrome or stable) and the study in which individuals were initially recruited.

Abbreviations and acronyms

CMA	Coronary Microcalcification Activity
CT	Computed Tomography
MACE	Myocardial Adverse Cardiovascular Events
PET	Positron Emission Tomography
SD	Standard Deviation

SUV	Standard Uptake Value
TBR	Maximum Target to Background Ratio
^{18}F-NaF	^{18}F-sodium Fluoride

Introduction

Despite improvements in therapies for atherosclerotic disease, myocardial infarction remains a leading cause of death worldwide. Robust tools to identify patients at risk of myocardial infarction would be extremely valuable as they could facilitate the targeted application of novel or intensive therapies to patients at the highest risk of events or down escalation of therapy in patients at low risk. However, to date, risk prediction in patients with established coronary artery disease has proven challenging. Current approaches are based around clinical risk scores, anatomic assessments of coronary artery calcification and the severity of obstructive coronary stenoses (1). These approaches have shown limited predictive value in patients with established coronary artery disease and there is growing interest in novel risk stratification methods, including assessments of atherosclerotic disease activity (2), that might be used to target expensive yet effective new treatments to patients at highest risk.

Advanced positron emission tomography (PET) imaging can provide assessment of disease activity in the coronary arteries to complement the anatomic plaque imaging provided by computed tomography (CT). The PET tracer ^{18}F-sodium fluoride (^{18}F-NaF) is a marker of developing microcalcification and calcification activity across multiple different cardiovascular disease states (3). In coronary and carotid atherosclerosis, ^{18}F-NaF localizes to culprit plaques following myocardial infarction and stroke as well as to plaques with multiple adverse characteristic in patients with stable disease (4-6). Moreover, coronary ^{18}F-NaF uptake has demonstrated its ability to predict disease progression and change in coronary calcium score, similar to results in other cardiovascular conditions (7-9). While coronary ^{18}F-NaF uptake appears to provide a marker of atherosclerosis disease activity, the prognostic significance of increased coronary ^{18}F-NaF activity is unknown.

In this study, we investigated whether coronary ^{18}F-NaF PET uptake predicts future myocardial infarction and MACE in patients with established coronary artery disease, and whether it can provide additional prognostic information over and above current methods of risk stratification including clinical risk scores, coronary calcium scoring and the severity of obstructive coronary artery disease.

Methods

Study Design and Participants

Patients with established coronary artery disease undergoing hybrid coronary ^{18}F-NaF PET and contrast CT angiography at the Edinburgh Heart Centre and Cedars-Sinai Medical Center within prospective observational research studies were included in the current post-hoc analysis (NCT01749254, NCT02110303, NCT02607748) (4,10). The study cohort comprised patients with recent myocardial infarction or established stable angina pectoris undergoing elective invasive coronary angiography (inclusion and exclusion criteria have been presented in the Online Appendix). All patients underwent a comprehensive baseline clinical assessment including evaluation of their cardiovascular risk factor profile. In particular, REACH [Reduction of Atherothrombosis for Continued Health] and SMART [Secondary Manifestations of Arterial Disease] risk scores were calculated (Online Appendix). Both these scores were created specifically to predict risk in patients with established coronary artery disease (1,11). Patients also underwent hybrid ^{18}F-NaF PET imaging alongside coronary CT calcium scoring and coronary CT angiography. Studies were conducted with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and with the written informed consent of each participant.

[18]F-Sodium Fluoride and CT imaging

Acquisition and reconstruction

All patients underwent ^{18}F-NaF PET on hybrid PET/CT scanners (128-slice Biograph mCT, Siemens Medical Systems, Knoxville, USA or Discovery 710 GE Healthcare, Milwaukee, WI, USA) using harmonized imaging protocols 60 min following intravenous ^{18}F-NaF administration. During a single imaging session, we acquired a non-contrast CT attenuation correction scan followed by a 30-min PET emission scan in list mode. The electrocardiogram (ECG)-gated list mode dataset was reconstructed using a standard ordered expectation maximization algorithm with time-of-flight, and point-spread-function correction. Using 4 cardiac gates, the data were reconstructed on a 256x256 matrix (with 75 or 47 slices using 2 iterations, 21 subsets and 5-mm Gaussian smoothing or 4 iterations, 24 subsets and 5-mm gaussian smoothing for Biograph and Discovery respectively). Immediately after the PET scan, a low dose non-contrast ECG-gated CT for calculation of the coronary calcium score was performed. Subsequently, a contrast-enhanced, ECG-gated coronary CT angiogram was obtained in mid-diastole on the same PET/CT system without repositioning the patient. To compensate for coronary motion associated with heart contraction, we performed cardiac motion correction of the PET/CT images (Online Appendix) (12,13).

Image analysis

Computed Tomography

The coronary artery calcium score was measured in Agatston units (AU) using clinical software (NetraMD, ScImage, Los Altos, CA, USA). The presence of coronary atherosclerosis, and the extent and severity of obstructive coronary artery disease, was evaluated on contrast-enhanced CT angiography by defining the segment involvement score; the number of vessels with >50% luminal stenosis; and the modified Duke coronary artery disease index (combining the extent,

severity, and location of coronary stenoses) (14). Multivessel coronary artery disease was defined as at least 2 major epicardial vessels with any combination of either >50% stenosis, or previous revascularization.

^{18}F-Sodium Fluoride

We used a dedicated software package for coronary PET image analysis (FusionQuant, Cedars-Sinai Medical Center, Los Angeles). PET and CT angiography reconstructions were reoriented, fused and systematically co-registered in 3 orthogonal planes (15). We used two methods to evaluate coronary ^{18}F-NaF activity: the maximum target to background (TBR) approach (standard quantification) which relies on visual detection of lesions with increased tracer uptake; and the newly developed whole-coronary total microcalcification activity method (novel quantification) (4,16).

Target to Background Ratio quantification

On co-registered PET and CT angiography images, for a signal to be co-localized to a coronary artery, an atherosclerotic plaque had to be present on the CT angiogram and the increased pattern of radiotracer had to arise from the coronary artery and follow its course in three dimensions on 3-orthogonal views (3). In all plaques meeting these criteria, maximum standardized uptake values (SUVmax) were measured within manually drawn regions of interest. TBR values were calculated by dividing the coronary SUVmax by the blood pool activity measured in the right atrium (mean SUV in cylindrical volumes of interest at the level of the right coronary artery ostium: radius 10 mm and thickness 5 mm).

Blood clearance correction

To offset for variation in the delay between tracer injection and scanning, which has a major impact on blood pool activity, we used a recently validated correction factor to harmonize the

background activity to a reference 60-minute injection-to-acquisition interval (Online Appendix) (17).

Coronary microcalcification activity (CMA) quantification

We used a recently described measure of coronary ^{18}F-NaF uptake, that quantifies PET activity across the entire coronary vasculature based upon analysis widely employed in oncology and cardiac sarcoidosis (16,18,19). First, we automatically extracted whole-vessel tubular and tortuous 3D volumes of interest from CT angiography datasets (Central Illustration, Online Appendix). These encompass all the main epicardial coronary vessels and their immediate surroundings (4-mm radius) facilitating per-vessel and per-patient uptake quantification. Within such volumes of interest, we measured the coronary microcalcification activity (CMA)— representing the overall disease activity in the vessel and based upon both the volume and intensity of ^{18}F-NaF PET activity within it (similar in principle to the Agatston score used for CT calcium scoring). CMA was defined as the integrated activity in SUV units exceeding the corrected background blood-pool mean SUV + 2 standard deviations (right atrium activity). The per-patient CMA was defined as the sum of the per-vessel CMA values.

Clinical Follow-up

The primary endpoint of the study was fatal or non-fatal myocardial infarction. The secondary endpoint was major adverse cardiovascular events (MACE), defined as myocardial infarction, stroke, delayed revascularization (more than 6 months after PET/CT) and cardiovascular death. Outcome information including invasive coronary angiography and coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft surgery) were obtained from the local and national healthcare record systems that integrates primary and secondary health

care records. Categorization of these outcomes was performed blinded to the coronary PET or other study data. Outcome data were collected in July 2019.

Statistical analysis

We assessed the distribution of data with the Shapiro-Wilk test. Continuous parametric variables were expressed as mean (SD) and compared using Student's t tests. Non-parametric data were presented as median [Q1-Q3] and compared using Mann-Whitney U test. Fisher's exact test or chi-squared test was used for analysis of categorical variables. We used the receiver-operating characteristic (ROC) analysis and pairwise comparisons according to DeLong *et al* to compare areas under the curves. Kaplan-Meier curves were used to elucidate the survival distributions with regard to myocardial infarction and MACE. Differences in the outcome of patients with and without ^{18}F-NaF coronary activity exceeding the threshold derived from the ROC using Youden's index were assessed using the log-rank test. A Cox proportional hazard regression with adjustment for potential confounders was performed to determine the predictors of worse outcome. Statistical analysis was performed with SPSS version 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). A two-sided p<0.05 was considered statistically significant.

Results

Patients

The study population comprised 293 patients (84% males, mean age: 65±9 years). All participants had established coronary artery disease, the majority (n=232) had stable disease and the remaining 61 individuals were recruited and imaged (14 [10-19)] days) following recent (7 [3-11] days) myocardial infarction (Online Appendix). Patients had advanced coronary atherosclerosis with a high burden of cardiovascular risk factors (hypertension 60%,

hyperlipidemia 88%, tobacco use 67%, REACH clinical risk scores of 13 [11-15], SMART

clinical risk scores of 18 [13-26]), widespread utilization of secondary preventative therapies

(statin 90%, anti-platelet therapy 92%, ACE inhibitor or angiotensin receptor blockers 67%) and

high rates of prior revascularization (n=237, 81%). None of the patients were taking PCSK9

inhibitor or interleukin 1-beta inhibitor therapy. On invasive angiography, 87 (30%) individuals

had single vessel obstructive disease, 191 (65%) had multi-vessel obstructive coronary artery

disease, and 18 (6%) had left main stem involvement.

Computed Tomography

Patients had advanced coronary artery disease on CT. The median CT calcium score was 334

[76-804], 59 (20%) subjects had a calcium score > 1000, 133 (45%) patients had a score > 400,

and only 84 (29%) presented with a score <100. On coronary CT angiography, the overall

median segment involvement score was 5 [3-7] with three-quarters of patients (n=218, 74%)

having at least 4 segments involved (Online Appendix). The median modified Duke index was 4

[3-5].

Positron Emission Tomography

On visual analysis of coronary PET, we identified increased tracer activity in 208 (70.9%)

patients. Across the entire cohort, we found a median TBR of 1.22 [1.10-1.42]. Compared to

those without uptake, patients with increased coronary ^{18}F-NaF uptake had higher SMART risk

scores (17 [13-23] vs 19 [13-27], p=0.029), and higher coronary calcium scores (184 [50-528] vs

371 [102-974] AU, p=0.0031), but there was no difference in the presence or severity of

obstructive coronary stenoses (all p>0.10).

Assessing whole vessel microcalcification activity, 203 (69.3%) patients presented with CMA>0. The median CMA value was 0.66 [0-2.84]. Again, we observed that patients with a CMA>0 had higher SMART risk scores (17 [13-23] vs 19 [13-27], p=0.028) and increased coronary calcium scores (378 [103-993] vs 179 [48-529], p=0.003) than subjects with CMA=0, but there was no difference in the presence or severity of obstructive coronary stenoses (all p>0.10; Online Appendix).

Clinical Outcomes

Over the 42 [31-49] months of follow-up, 20 subjects experienced a fatal (n=3) or non-fatal (n=17) myocardial infarction. Seven of these occurred in patients imaged following an acute coronary syndrome who had a median time from PET/CT to recurrent myocardial infarction of 12 (6-15) months. During follow-up a total of 40 patients suffered a MACE event (20 myocardial infarctions, 12 strokes, 3 cardiovascular deaths and 5 cases of delayed revascularization)

Primary endpoint: fatal or non-fatal myocardial infarction

Patients who experienced myocardial infarction during follow-up had higher TBR values than those who did not (1.40 [1.28-1.77] versus 1.21 [1.09-1.40], p=0.006) and CMA (3.05 [1.62-5.25] versus 0.46 [0-2.47], p=0.002; Figure 1). Indeed, all the patients who had an infarct had increased coronary ^{18}F-NaF PET uptake at baseline (CMA > 0). Interestingly, patients who experienced a fatal or non-fatal myocardial infarction did not have increased clinical risk scores (REACH: 13 [11-15] versus 13 [11-15], p=0.79; SMART 20 [13-28] versus 18 [13-26], p=0.52) nor coronary calcium scores (397 [39-1456] versus 331 [76-775] AU, p=0.60) compared to patients who did not have an infarct. Moreover, they did not have an increased prevalence of obstructive coronary artery disease (segment involvement score 6 [4-8] versus 5 [3-7], p=0.25), multivessel coronary disease (70% versus 65%, p=0.64) nor previous coronary stents (75%

versus 74%, p=1.00). In patients who had a fatal or non-fatal myocardial infarction, 30% had a coronary calcium score <100 AU, 20% were within the 100-399 AU range, 20% were within the 400-999 AU range and 30% had a coronary calcium score >1000 AU (Figures 2 & 3). Only 12% (7/59) of patients with coronary calcium score >1000 AU experienced myocardial infarction (Online Appendix).

On ROC analysis, both CMA and TBR showed a greater area under the curve for the prediction of myocardial infarction than coronary calcium scores, or the REACH and SMART clinical risk scores (Online Appendix). In order to generate distinct clinical risk groups, we dichotomized the population according to their coronary ^{18}F-NaF uptake and derived the optimal TBR and CMA cutoffs for event prediction using the Youden's index. A threshold of 1.56 for CMA achieved a specificity and sensitivity of 66% and 80% for the primary endpoint. A threshold of 1.28 for TBR achieved a specificity of 63% and sensitivity of 80% (Table 1). On univariable Cox proportional regression, both CMA >1.56 (hazard ratio (HR) 7.30, 95% confidence interval (CI) 2.44-21.84; p<0.001) and TBR >1.28 (HR 6.16, 95% CI 1.06-18.42; p=0.001) emerged as predictors of fatal or non-fatal myocardial infarction. Importantly, these associations persisted on multivariable analysis after adjustments for gender, comorbidities (presence of hypertension, hyperlipidemia, diabetes, smoking), the segment involvement score, number of coronary stents, multivessel coronary artery disease, coronary calcium score, SMART and REACH risk scores, initial patients presentation (acute coronary syndrome or stable coronary artery disease) and the study in which individuals were initially recruited (Figure 4). Indeed patients with CMA>1.56 had an adjusted hazard ratio of 7.1 (95% CI 2.2 to 25.1; p=0.003) for the primary end point, whilst patients with a TBR >1.28 had an adjusted hazard ratio of 4.6 (95% CI 1.4 to 14.4, p=0.013; Table 2). Similar results were observed when both

CMA and TBR were considered as continuous variables, with both again emerging as the only independent predictors of fatal or non-fatal myocardial infarction on Cox modelling (Online Appendix). In contrast, the number of stenosed vessels, the modified Duke index, age, and the SMART and REACH risk scores did not emerge as predictors of fatal non-fatal myocardial infarction on univariable Cox modelling (all p>0.1, Online Appendix). Coronary calcium score was a predictor of events on univariable but not multivariable analysis (Table 2). Despite low statistical power when patients with acute myocardial infarction and stable subjects were considered separately, the AUCs on receiver-operator-characteristic curve analyses remained numerically similar (Online Appendix).

Secondary Endpoint: Major Adverse Cardiovascular Events

Patients with MACE had higher CMA (1.9 [1.65-4.76] versus 0.51 [0-2.42], p=0.0098) and an apparent trend for higher TBR values (1.34 [1.13-1.54] versus 1.22 [1.10-1.40], p=0.073) than patients without MACE. There were no differences in the extent of obstructive coronary artery disease on CT angiography (the segment involvement score, the modified Duke index, presence of multivessel disease or coronary stents) nor cardiovascular risk scores and co-morbidities in patients with and without MACE (Online Appendix). Similarly, there was no difference in coronary calcium scores 195 [50-1126] versus 344 [81-801] AU, p=0.50). Only 17% (10/59) of patients with a coronary calcium score >1000 AU experienced MACE.

On univariable Cox proportional regression, both CMA>1.56 and TBR>1.28 were predictors of MACE (HR 2.3, 95% CI 1.2-4.3, p=0.01 and HR 2.1, 95% CI 1.1-3.9, p=0.02). On multivariable analysis after adjustments for age, gender, comorbidities (presence of hypertension, hyperlipidemia, diabetes, smoking), the segment involvement score, number of coronary stents multivessel coronary artery disease, coronary calcium score and the REACH and

SMART risk scores, CMA remained the only independent predictor of MACE (HR 2.1, 95% CI 1.1-4.1, p=0.030; Figure 4). When CMA and TBR were considered as continuous variables, these two measurements emerged as the only predictors of MACE on Cox modelling (Online Appendix).

In contrast, coronary calcium score exceeding 1199 AU (HR 1.9, 95% CI 0.9-4.0, p=0.07) , the modified Duke index (HR 1.2, 95% CI 0.9-1.6, p=0.14), the REACH (HR 1.7, 95% CI 0.5-5.5, p=0.38) and SMART (HR 1.5, 95% CI 0.8-2.8, p=0.23) risk scores were not predictors of MACE on univariable analysis.

Discussion

In this two-center multimodality imaging study, we have demonstrated for the first time that coronary ^{18}F-NaF PET is a powerful prognostic tool for predicting myocardial infarction in patients with advanced established coronary artery disease. In a comprehensive analysis, we show that both ^{18}F-NaF TBR values and whole vessel CMA emerge as powerful independent predictors of myocardial infarction outperforming all other established predictors including the presence of co-morbidities, the REACH and SMART risk scores, coronary calcium scoring and the presence, severity and extent of coronary artery disease. Our data therefore highlight the added prognostic value that assessments of disease activity can provide and confirm the potential of ^{18}F-NaF PET to improve the risk stratification of patients with established CAD, a group in whom prediction of events has previously proved challenging.

[18]F-NaF PET provides an assessment of calcification activity across multiple different cardiovascular disease states including aortic stenosis, mitral annular calcification, abdominal aortic aneurysm, erectile dysfunction and bioprosthetic valve degeneration (7,20). In each condition, it is associated with vascular injury, disease activity and future disease progression.

This is also the case in coronary atherosclerosis. Increased ^{18}F-NaF uptake is associated with culprit coronary plaques in patients with myocardial infarction and adverse plaque features in patients with apparently stable disease (4). Moreover, similar to other cardiovascular conditions, baseline coronary ^{18}F-NaF activity predicts the future progression of coronary calcium scores, confirming its status as a marker of disease activity (5,6). While there is major interest in using markers of atherosclerotic disease activity to improve patient assessment and risk stratification, this is the first study to demonstrate that increased ^{18}F-NaF activity provides powerful prediction of future myocardial infarction. Indeed, this technique outperformed all the other commonly used predictors of events in patients with established coronary artery disease including two established clinical risk scores designed for this patient population, co-morbidities, coronary calcium scoring, and the presence and severity of obstructive coronary artery disease. ^{18}F-NaF might therefore provide an important clinical tool in a patient population in whom risk stratification is currently suboptimal. A CMA >1.56 was associated with a >7-fold risk of myocardial infarction. This was despite almost universal prescription of aspirin, statins and other secondary preventative therapies. These patients might therefore be suitable for advanced medical therapies including PCSK9 or interleukin 1-beta inhibition, with ^{18}F-fluoride PET providing the risk stratification tool that many have advocated for as a means of targeting these expensive drugs to those patients at greatest risk. In the wake of the ISCHEMIA trial this approach might also help select patients who would benefit from revascularization (21). Of equal importance, patients without coronary ^{18}F-NaF uptake and a CMA=0 had an excellent prognosis with no myocardial infarctions observed in this group despite their advanced coronary artery disease. In these patients with dormant coronary artery disease (a third of the population studied), further intensification of medical therapy might not be warranted, nor might they benefit on prognostic

grounds from complex revascularization such as multivessel percutaneous intervention or coronary artery bypass grafting. Further research is required to investigate these important clinical questions.

Our data demonstrating the modest predictive value of cardiovascular risk scores, coronary calcium scoring and obstructive coronary artery disease in patients with advanced established coronary artery disease is consistent with the recent literature. The diagnostic performance of the REACH and SMART risk scores was poor in several recent studies (C-statistic of 0.53 and 0.54 respectively (1,22). While coronary calcium scoring provides powerful prognostic information in asymptomatic individuals and those presenting with chest pain, its prognostic capability has been disappointing in other studies of patients with established advanced coronary artery disease (23,24). In line with recent literature, the presence and extent of obstructive coronary artery disease was also not a marker of adverse events in our study (25,26).

Our study has notable strengths. We have focused our analysis on patients with advanced established coronary artery disease for whom we lack robust methods for risk stratification and showed that ^{18}F-NaF PET has the potential to fulfill this unmet clinical need. We utilized state-of-the-art ^{18}F-NaF PET imaging, employing the latest advances in image acquisition and motion correction (14). We also employed a novel quantification technique, CMA, that measures ^{18}F-NaF uptake along the course of the entire coronary vasculature and therefore provides a more complete summative assessment of disease activity than the TBR values derived from visually defined hot spot assessments (16). While both standard TBR values and CMA emerged as independent predictors of myocardial infarction, CMA demonstrated a superior hazard ratio for this endpoint, and was also the only independent predictor of MACE. CMA would therefore

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appear to hold advantages as a method for quantifying overall coronary ^{18}F-NaF uptake and disease activity.

Limitations

Our study has some limitations. It is a post-hoc analysis of data collected for prospective observational studies. While all the subjects had advanced established coronary artery disease, we have included patients with both stable and unstable coronary artery disease thereby increasing the heterogeneity of the analyzed cohort. Similar results were, however, observed when patients with unstable coronary artery disease were excluded from the analysis (online appendix). Our data therefore require confirmation in large prospective studies. Indeed, we are currently completing recruitment for the Prediction of Recurrent Events With ^{18}F-Fluoride (PREFFIR) study which will prospectively investigate the ability of ^{18}F-NaF coronary PET to predict recurrent events in patients with multi-vessel disease and recent myocardial infarction. While performing a CT angiogram alongside the ^{18}F-NaF PET scan incurs a modest additional dose of radiation, this is currently essential for accurate image co-registration, interpretation and analysis (15). Although we have shown that delayed ^{18}F-NaF imaging may improve image quality, in this study participants underwent PET imaging 1 h after tracer injection (27). The potential prognostic benefits of delaying image acquisition therefore remain to be evaluated.

Conclusions

^{18}F-NaF PET is a determinant of disease activity in the coronary arteries and a powerful prognostic technique to predict myocardial infarction in patients with advanced established coronary artery disease. Further studies are required to confirm our findings and to investigate how best to use this technique to improve patient risk stratification and to guide the use of advanced therapeutic interventions.

Clinical Perspective:

COMPETENCY IN MEDICAL KNOWLEDGE: ^{18}F-NaF coronary PET provides an assessment of disease activity in coronary atherosclerosis and powerful independent prediction of myocardial infarction in patients with established coronary artery disease.

TRANSLATIONAL OUTLOOK: ^{18}F-NaF PET holds promise in risk stratifying patients with established coronary artery disease and as a tool to up titrate therapy (e.g. PCSK9 and interleukin 1-beta inhibition) in those at high risk, whilst down grading therapy and intervention in patients at low risk.

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Figure Legends

Figure 1. Coronary disease activity and plaque burden in patients with and without future myocardial infarction. Coronary microcalcification activity (CMA, top row), maximum target to background ratios (TBR, middle row) and the coronary calcium scores (CCS, bottom row) in patients with and without myocardial infarction during follow-up. For the Kaplan-Meier curves patients were dichotomized according to thresholds derived from receiver operator curves using the Youden's index: CMA=1.56, TBR=1.28 and coronary calcium score = 1199 Agatston-units.

Figure 2. Case examples of ^{18}F-sodium fluoride positron emission tomography in patients with established coronary artery disease and myocardial infarction during follow-up. Hybrid CT angiography and ^{18}F-NaF positron emission tomography of coronary arteries in: **(A)** a 56-year-old male who demonstrated increased ^{18}F-NaF uptake in the RCA at baseline and presented with an inferior ST-segment elevation myocardial infarction and occlusion of the RCA during follow-up; **(B)** a 52-year-old male who demonstrated increased ^{18}F-NaF uptake in the LCx at baseline and presented with a lateral non-ST-segment elevation myocardial infarction during follow-up; **(C)** a 60-year-old female who showed increased ^{18}F-NaF uptake in the proximal RCA and presented with an inferior non-ST-segment elevation myocardial infarction during follow-up. LAD–left anterior descending, LCx–left circumflex, RCA–right coronary artery.

Figure 3. ^{18}F-sodium fluoride positron emission tomography in the prediction of myocardial infarction in patients with established coronary artery disease. In patients with established atherosclerosis the coronary microcalcification activity (as a marker of ^{18}F-NaF activity across the coronary vasculature) had a significantly larger area under the receiver operator curve than the coronary calcium score (non-contrast CT), the modified Duke index

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(contrast CT angiography) or the REACH score (patient clinical data).AU-Agatston units, CMA–coronary microcalcification activity, REACH-Reduction of Atherothrombosis for Continued Health

Figure 4. Predictors of myocardial infarction on Cox proportional hazards modelling. Forest plots of hazard ratios derived from multivariable modelling with 95% confidence intervals for the coronary microcalcification activity (CMA) (A) and the target to background ratio values (B) along with covariates: coronary calcium scores, SIS, REACH score, SMART score, total number of implanted coronary stents, presence of multivessel coronary artery disease, age, gender, hyperlipidemia, hypertension, diabetes, smoking. CMA–coronary microcalcification activity, REACH-Reduction of Atherothrombosis for Continued Health, SMART - Secondary Manifestations of Arterial Disease, SIS–segment involvement score, TAG-triacylglycerides, TBR–target to background ratio

Central Illustration. **^{18}F-sodium fluoride positron emission tomography as a marker of disease activity in the coronary arteries is a predictor of fatal or non-fatal myocardial infarction (MI) in patients with established coronary artery disease.** ^{18}F-fluoride PET can be used to measure disease activity across the coronary vasculature and to stratify patients into those with no, low and high disease activity. Patients with high disease activity (coronary microcalcification activity (CMA) >1.56) demonstrate a >7-fold risk of myocardial infarction. These patients might therefore be suitable for advanced medical therapies including PCSK9 or interleukin 1-beta inhibition, with ^{18}F-fluoride PET used for targeting these expensive drugs to patients at greatest risk. Patients without coronary ^{18}F-NaF uptake (CMA=0) have an excellent prognosis with no myocardial infarctions observed during follow-up despite advanced coronary artery disease. In these patients with dormant coronary artery disease (a third of the population

studied), further intensification of medical therapy might not be warranted, nor might they

benefit on prognostic grounds from complex revascularization such as multivessel percutaneous

intervention or coronary artery bypass grafting.

Table 1. Baseline Characteristics of Study Participants. Comparison of patients with coronary microcalcification activity (CMA) ≥1.56 vs <1.56, with Target to Background ratio (TBR) ≥1.28 vs <1.28 and coronary calcium score ≥1199 vs <1199.

	CMA			TBR			CCS		
	≥ 1.56 (n=109)	< 1.56 (n=184)	P	≥1.28 (n=113)	<1.28 (n=180)	P	≥1199 (n=45)	<1199 (n=248)	P
Age in years, mean (SD)	67 (8)	64 (9)	**0.0047**	67 (8)	63 (9)	**0.0001**	68 (8)	64 (9)	**0.006**
Men, n (%)	97 (89%)	148 (80%)	0.071	103 (91%)	142 (79%)	**0.006**	44 (98%)	201 (81%)	**0.004**
Body-mass index (kg/m2), mean (SD)	28 (5)	30 (5)	**0.024**	29 (6)	29 (5)	1.00	30 (5)	29 (5)	0.22
Systolic blood pressure (mm Hg), mean (SD)	142 (21)	141 (20)	0.68	142 (20)	141 (20)	0.68	143 (15)	141 (21)	0.54
Diastolic blood pressure (mm Hg), mean (SD)	79 (12)	80 (11)	0.46	78 (11)	80 (12)	0.15	78 (11)	80 (11)	0.26
Cardiovascular history, n (%)									
H of ACS	53 (48.6%)	108 (58.7%)	0.11	58 (51.3%)	103 (57.2%)	0.34	25 (55.6%)	136 (54.8%)	1.00
H of PCI	64 (58.7%)	119 (64.7%)	0.32	69 (61.1%)	114 (93.3%)	0.71	23 (51.1%)	160 (64.5%)	0.10
H of CABG	20 (18.3%)	28 (15.2%)	0.52	21 (18.6%)	27 (15.0%)	0.44	23 (51.1%)	25 (10.1%)	**0.0001**
H of angina	60 (55.0%)	76 (41.3%)	**0.029**	57 (50.4%)	79 (43.9%)	0.28	30 (66.7%)	106 (42.7%)	**0.003**
CVA/TIA	3 (2.8%)	6 (3.3%)	1.000	3 (2.7%)	6 (3.3%)	1.00	1 (2.2%)	8 (3.2%)	1.00
Comorbidities/risk factors, n (%)									
HTN	71 (65.1%)	103 (55.9%)	0.14	76 (67.3%)	98 (54.4%)	**0.038**	30 (66.7%)	144 (58.1%)	0.32
HPL	97 (89.0%)	160 (86.9%)	0.71	101 (89.3%)	156 (86.7%)	0.58	40 (88.9%)	217 (87.5%)	1.00
DM	26 (23.9%)	35 (19.0%)	0.37	26 (23.0%)	35 (19.4%)	0.46	13 (28.9%)	48 (19.4%)	0.16
Current smoking	20 (18.3%)	38 (21.1%)	0.65	21 (18.6%)	37 (20.6%)	0.76	8 (17.8%)	50 (20.2%)	0.84
Ex-smoker	44 (40.3%)	93 (51.7%)	0.12	51 (45.1%)	86 (47.8%)	0.72	19 (42.2%)	118 (47.6%)	0.52
Atrial fibrillation	4 (3.7%)	6 (3.3%)	1.00	5 (4.4%)	5 (2.8%)	0.52	2 (4.4%)	8 (3.2%)	0.65
Peripheral vascular disease	4 (3.7%)	12 (6.5%)	0.43	4 (3.5%)	12 (6.7%)	0.30	8 (17.8%)	8 (3.2%)	**0.0008**
Medications, n (%) *									
Aspirin	101 (92.7%)	167 (90.7%)	0.67	107 (94.7%)	161 (89.4%)	0.14	41 (91.1%)	227 (91.5%)	1.00
PY12 antagonist	19 (17.4%)	26 (14.1%)	0.50	21 (18.6%)	24 (13.3%)	0.25	5 (11.1%)	40 (16.1%)	0.50
Statin	102 (93.6%)	160 (86.9%)	0.08	103 (91.2%)	159 (88.3%)	0.56	42 (93.3%)	220 (88.7%)	0.44
Beta Blocker	72 (66.1%)	124 (67.4%)	0.90	77 (68.1%)	119 (66.1%)	0.80	32 (71.1%)	164 (66.1%)	0.61
ACEI/ARB	76 (69.7%)	121 (65.7%)	0.61	81 (71.7%)	116 (64.4%)	0.20	38 (84.4%)	159 (64.1%)	**0.009**
Insulin	1 (0.9%)	3 (1.6%)	1.00	1 (0.9%)	3 (1.7%)	1.00	0	4 (1.4%)	1.00
Oral diabetic medications	17 (15.6%)	31 (16.8%)	0.87	19 (16.8%)	29 (1.6%)	0.87	8 (17.8%)	40 (16.1%)	0.83
CCB	23 (21.1%)	40 (21.7%)	1.00	27 (23.9%)	36 (20.0%)	0.47	12 (26.7%)	51 (20.6%)	0.43
Diuretics	7 (6.4%)	31 (16.8%)	**0.028**	7 (6.2%)	31 (17.2%)	**0.007**	7 (15.6%)	31 (12.5%)	0.63
Biomarkers, median (IQR)									
Total Cholesterol (mmol/L)	4.0 (3.5-4.7)	4.1 (3.6-4.8)	0.41	4.0 (3.5-4.8)	4.1 (3.6-4.7)	0.63	4.2 (3.5-4.9)	3.8 (4.1-4.7)	0.53
LDL (mmol/L)	1.9 (1.3-2.5)	1.9 (1.2-2.4)	0.75	1.7 (1.2-2.5)	2.1 (1.4-2.4)	0.21	2.2 (1.2-2.7)	1.9 (1.2-2.4)	0.12
HDL (mmol/L)	1.2 (1.0-1.7)	1.2 (1.0-1.8)	0.76	1.2 (1.0-1.7)	1.2 (1.0-1.7)	0.69	1.2 (1.0-1.7)	1.2 (1.0 −1.7)	0.74
TAG (mmol/L)	1.5 (1.1-2.5)	1.6 (1.1-2.2)	0.87	1.5(1.0-2.5)	1.5 (1.1-2.2)	0.87	1.4 (1.2-2.0)	1.6 (1.1-2.4)	0.37

Creatinine (µmol/L)	80 (70-94)	77 (70-89)	0.42	78(70-88)	78(70-92)	0.95	80 (70-91)	77 (70-90)	0.48
CAD, n (%)									
Non-obstructive disease (<50%)	5 (4.6%)	10 (5.4%)	0.79	2 (1.8%)	13 (7.2%)	0.05	1 (2.2%)	14 (5.6%)	0.48
Single vessel disease	31 (28.4%)	56 (30.4%)	1.00	29 (25.7%)	58 (32.2%)	0.24	8 (17.8%)	79 (31.9%)	0.08
Two vessel disease	37 (33.9%)	73 (39.7%)	0.38	44 (38.9%)	66 (36.7%)	0.71	12 (26.7%)	98 (39.5%)	0.13
Three vessel disease	36 (33.0%)	45 (24.5%)	0.10	38 (33.6%)	43 (23.9%)	0.081	24 (53.3%)	57 (23.0%)	**0.0001**
LMS involvement	7 (6.4%)	7 (3.8%)	0.40	7 (6.2%)	7 (3.9%)	0.41	7 (15.6%)	7 (2.8%)	**0.002**
Coronary Stent, n (%)	83 (76.1%)	135 (73.4%)	0.68	85 (75.2%)	133 (73.9%)	0.89	24 (53.3%)	194 (78.2%)	**0.0013**
Segment involvement score. Median (IQR)	6 (4-8)	5 (3-7)	**0.008**	4 (6-8)	5(3-7)	**0.002**	7 (6-9)	5 (3-7)	**<0.0001**
SIS breakdown, n (%) 0-1	4 (3.7%)	16 (8.7%)	0.15	3 (2.7%)	17 (9.4%)	**0.030**	1 (2.2%)	19 (7.7%)	0.33
2-3	19 (16.4%)	36 (19.6%)	0.76	14 (12.3%)	41 (22.8%)	**0.031**	0	55 (22.2%)	**0.0001**
4-5	23 (21.1%)	50 (27.2%)	0.27	29 (25.6%)	44 (24.4%)	0.89	5 (11.1%)	68 (27.4%)	**0.02**
>5	63 (57.8%)	82 (44.6%)	0.016	67 (59.3%)	78 (43.3%)	**0.009**	39 (86.7%)	106 (42.7%)	**0.0001**
CCS, median (IQR)	544 (184-1157)	201 (64-541)	**<0.0001**	498 (188-1089)	201 (59-558)	**<0.0001**	N/A	N/A	N/A
CCS, n (%) 0-99	21 (19.3%)	63 (34.2%)	**0.007**	20 (17.7%)	64 (35.6%)	**0.0009**			
100-399	24 (22.0%)	52 (28.3%)	0.27	25 (22.1%)	51 (28.3%)	0.2742			
400-999	27 (24.8%)	47 (25.5%)	1.00	33 (29.2%)	41 (22.8%)	0.2691			
>1000	37 (33.9%)	22 (12.0%)	**0.0001**	35 (31.0%)	24 (13.3%)	**0.0003**			
TBR, median (IQR)	1.45 (1.31-1.62)	1.13 (1.05-1.22)	**<0.001**	1.45 (1.35-1.62)	N/A	N/A	1.4 (1.23-1.62)	1.2 (1.1-1.4)	**<0.0001**
TBR≥1.28	86 (78.9%)	27 (14.7%)	**0.0001**	N/A	N/A	N/A	26 (57.8%)	87 (35.1%)	**0.005**
CCS>1199	27 (24.8%)	19 (10.3%)	**0.0015**	26 (23.0%)	20 (11.1%)	**0.0082**	N/A	N/A	N/A
Risk scores									
REACH score (IQR) CV event	13 (11-15)	13 (11-15)	0.075	14 (12-16)	12 (10-15)	**0.0039**	15 (13-17)	13 (11-15)	**<0.0001**
20-month risk of next CV event, % (IQR)	6.3 (4.7-8.5)	6.3 (4.7-8.5)	0.13	7.3 (4.7-9.2)	5.4 (4.0-8.5)	**0.0074**	8.5 (6.3-11.0)	5.4 (4.7-8.5)	**<0.0001**
REACH score (IQR) CV death	11 (10-13)	11 (9-13)	**0.015**	11 (10-13)	11 (9-12)	**0.0012**	12 (11-14)	11 (9-12)	**<0.0001**
20-month cardiovascular death, % (IQR)	1.8 (1.4-3.0)	1.8 (1.1-2.8)	**0.014**	1.8 (1.4-3.0)	1.5 (1.0-2.0)	**0.0011**	2.3 (1.8-3.8)	1.8 (1.1 -2.3)	**<0.0001**
Duke score	4 (3-5)	4 (3-5)	0.28	4 (3-5)	4 (3-5)	**0.0324**	5 (4-5)	4 (3-5)	**0.0024**
SMART risk score	21 (15-27)	17 (12-24)	**0.0050**	20 (14-28)	17 (13-24)	**0.0414**	24 (18-32)	17 (13-24)	**0.0002**
Outcomes									
Myocardial infarction	16 (14.7%)	4 (2.2%)	**0.0001**	16 (14.2%)	4 (2.2%)	**0.0002**	13 (15.6%)	7 (5.2%)	0.08
MACE	23 (21.1%)	17 (9.2%)	**0.008**	23 (20.4)	17 (9.4%)	**0.0078**	10 (22.2%)	30 (12.1%)	0.10
Stroke	3	9	**0.045**	3	9	**0.045**	1	11	**0.003**
Cardiovascular death	2	1	N/A	2	1	N/A	0	3	N/A
Delayed revascularization	2	3	N/A	2	3	N/A	0	5	N/A

ACEI/ARB – angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS – acute coronary syndrome, CABG – coronary artery bypass graft, CAD – coronary artery disease, CMA – coronary microcalcification activity, CVA - Cerebrovascular accident, MACE – major adverse cardiovascular event, PCI – percutaneous coronary intervention, REACH - Reduction of Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease, SIS – segment involvement score, TAG - triacylglycerides, TBR – target to background ratio, TIA - transient ischemic attack

*medications at the time of scan

Table 2. Uni- and multivariable Cox proportional regression models for prediction of myocardial infarction during follow-up.

	Coronary Microcalcification Activity >1.56		Target to background ratio >1.28		Coronary Calcium Score > 1199	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Model 1	7.30 (2.44-21.84)	<0.001	6.16 (1.06-18.42)	0.001	3.24 (1.29-8.11)	0.012
Model 2	7.20 (2.36-21.95)	0.001	5.94 (1.94-18.10)	0.002	-	
Model 3	6.66 (2.19-20.25)	0.001	5.57 (1.80-17.00)	0.003	2.65 (0.93-7.56)	0.069
Model 4	8.73 (2.44-31.29)	0.001	4.80 (1.54-14.93)	0.007	2.72 (0.90-8.21)	0.075
Model 5	8.91 (2.47-32.16)	0.001	4.83 (1.54-15.20)	0.007	-	
Model 6	8.12 (2.57-25.28)	p<0.001	4.30 (1.34-13.82)	0.014		
Model 7	7.10 (2.2-25.1)	0.003	4.6 (1.4-14.4)	0.013		

Model 1 – unadjusted; Model 2 – adjusted for Coronary Calcium Score; Model 3 – adjusted for segment involvement score, number of coronary stents, multivessel coronary artery disease; Model 4 – adjusted for segment involvement score, number of coronary stents,

multivessel coronary artery disease, age, gender, hyperlipidaemia, hypertension, diabetes, smoking; Model 5 – similar to Model 4 and additionally adjusted for coronary calcium scoring; Model 6 – similar to Model 5 and additionally adjusted for REACH and SMART risk scores. Model 7 – similar to model 6 and additionally adjusted for initial patient's presentation (stable vs acute myocardial infarction) and the study in to which the patient was initially recruited.
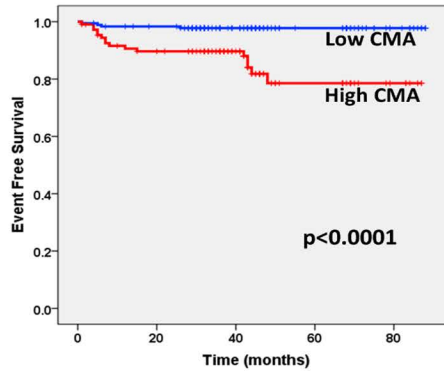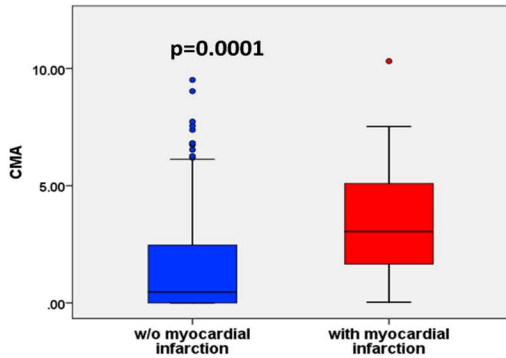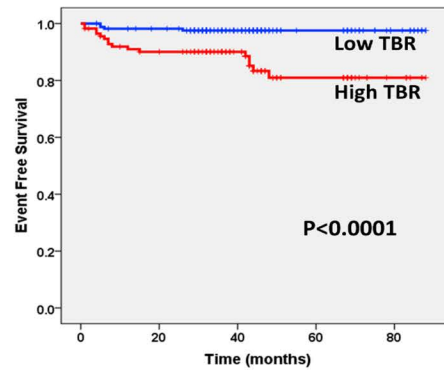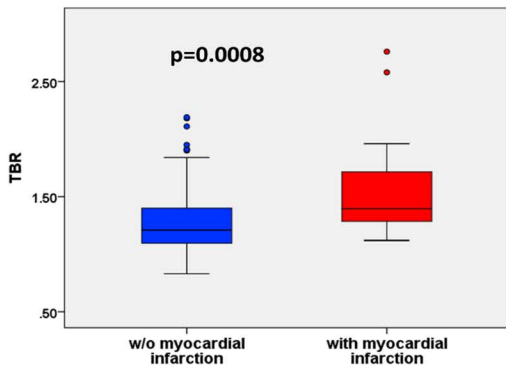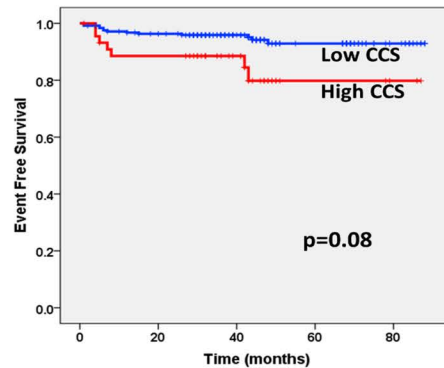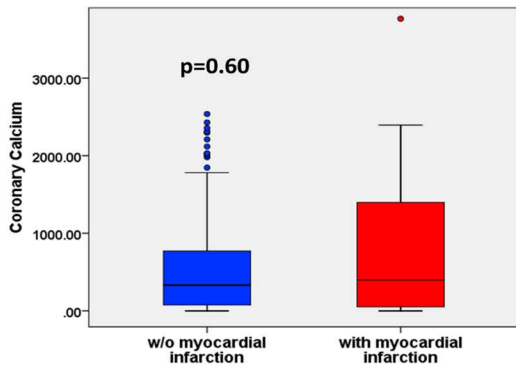
Figure 1

¹⁸F-NaF Coronary Uptake Predicts Myocardial Infarction

A

LAD   LCX   RCA   RCA
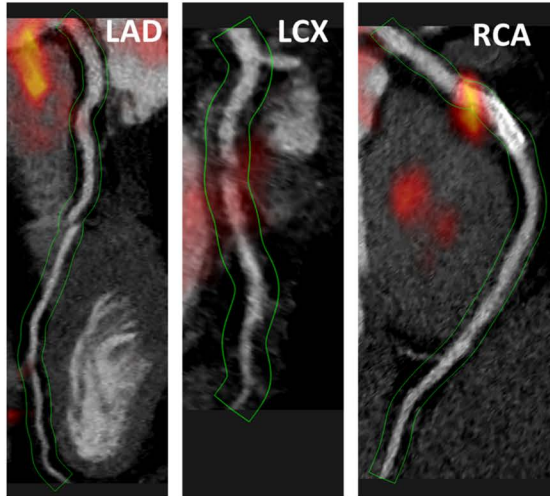
Coronary Calcium Score 6 AU
Coronary Microcalcification Activity 1.58
Target to Background Ratio 1.31

ST- segment elevation
Myocardial Infarction
3-years after ¹⁸F-NaF PET

B

LAD   LCX   RCA
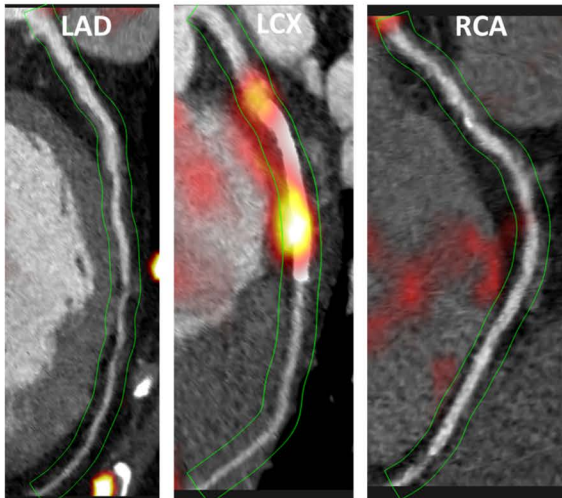
Coronary Calcium Score 26 AU
Coronary Microcalcification Activity 4.79
Target to Background Ratio 1.34

C

LAD   LCX   RCA
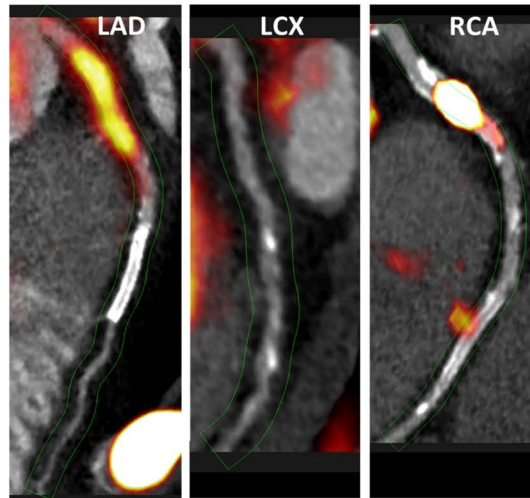
Coronary Calcium Score 306 AU
Coronary Microcalficiation Activity 4.36
Target to Background Ratio 1.82

Figure 2

Figure 3

A

	HR (95% CI)	p value
18F-NaF CMA	8.1 (2.6, 25.3)	<0.001
Calcium score	1.1 (0.9, 1.3)	0.41
SIS	1.2 (1.1, 1.4)	0.14
REACH score	1.1 (0.9, 1.3)	0.47
SMART score	1.1 (1.0, 1.1)	0.12
Age	0.9 (0.8, 0.9)	0.23
No. of Stents	1.0 (0.7, 1.4)	0.84
Multivessel CAD	1.0 (0.7, 1.4)	0.62
Gender	1.3 (0.4, 5.1)	0.67
Hyperlipidemia	1.2 (0.3, 5.4)	0.83
Hypertension	1.6 (0.7, 3.9)	0.30
Diabetes	1.8 (0.9, 6.3)	0.23
Smoking	0.5 (0.2, 1.5)	0.22

B

	HR (95% CI)	p value
18F-NaF TBR	4.3 (1.3, 13.8)	0.014
Calcium score	1.1 (0.9, 1.4)	0.38
SIS	1.2 (1.1, 1.4)	0.15
REACH score	1.1 (0.9, 1.3)	0.40
SMART score	1.1 (1.0, 1.1)	0.10
Age	0.9 (0.8, 0.9)	0.27
No. of Stents	1.0 (0.7, 1.4)	0.65
Multivessel CAD	1.0 (0.7, 1.4)	0.67
Gender	1.3 (0.6, 4.5)	0.58
Hyperlipidemia	1.2 (0.2, 5.8)	0.71
Hypertension	1.6 (0.7, 3.9)	0.33
Diabetes	1.8 (0.9, 7.8)	0.29
Smoking	0.5 (0.2, 1.5)	0.25

Figure 4

Online Appendix

18F-Sodium Fluoride Coronary Uptake Predicts Outcome in Patients with Coronary Artery Disease

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Brief Title: 18F-NaF uptake predicts outcome

Methods

REACH and SMART risk scores

REACH score. "REACH (REduction of Atherothrombosis for Continued Health) is an outpatient registry of patients with either stable symptomatic vascular disease (CAD, cerebrovascular disease, or peripheral artery disease) or with multiple atherosclerotic risk factors. The data collected from 3647 centers in 29 countries enabled establishing the REACH score which enables prediction of 20-month risk of a recurrent cardiovascular event (1). The REACH score calculator can be found at: https://www.u-prevent.com/en-GB/ReachCalculator/ReachCalculator

SMART Score. The SMART (Second manifestations of arterial disease) risk score estimates the 10-year risk for myocardial infarction, stroke or vascular death in individual patients with previous cardiovascular disease, including coronary artery disease, cerebrovascular disease, peripheral artery disease, abdominal aortic aneurysm and polyvascular disease. The SMART risk score was developed in a population of vascular patients in the Netherlands that were included in the Secondary Manifestations of Arterial Disease (SMART)-study (2). External validation and updating were performed in pooled trial cohorts of 18,436 vascular patients from W-Europe, S-Europe, Israel, USA, Canada, Mexico, S-Africa, Australia, and N-Zealand (3). The SMART score calculators can be found at: https://www.escardio.org/Education/ESC-Prevention-of-CVD-Programme/Risk-assessment/SMART-Risk-Score

Motion Correction

Coronary motion correction compensates for coronary artery motion by aligning all gates to the end-diastolic position and has already demonstrated its ability to reduce image noise and improve the reproducibility of acquired data (4). In the first step of motion correction, anatomical coronary artery data was extracted from coronary CT angiography by applying a vessel tracking algorithm based on Bayesian maximal paths (Autoplaque version 2.0). Secondly, a diffeomorphic mass-preserving image registration algorithm was used to align the 4 gates of PET data to the end-diastolic gate (FusionQuant Software, Cedars-Sinai Medical Center, Los Angeles). After motion correction, the 4 gates were summed together to build a motion-free image containing counts from the entire PET acquisition.

Blood clearance -correction

To offset for the variation in injection to scan delay that has a major impact on blood pool activity, we used a recently validated correction factor to harmonize the background activity to a reference 60-minute injection to acquisition interval

$$SUV_{Background\ corrected} = SUV_{Background} * e^{-0.004*(60-t)}$$

where t represents the injection-to-scan delay in minutes (5).

Results

Prediction of fatal and non-fatal myocardial infarction in stable patients and subjects with recent myocardial infarction

Over 42 [31-49] months of follow-up, 13 of 232 stable patients experienced a fatal or non-fatal myocardial infarction. Similar to the entire cohort patients, ^{18}F-NaF uptake measured using the CMA approach had the biggest area under the receiver operator curve (Online Figure 3). On Cox proportional modelling only CMA and coronary calcium scores emerged as predictors of outcome: HR=23.2 95% CI (3.0-178.5), p=0.003 and HR=5.7 95% CI (1.9-16.8), p=0.002 respectively. Importantly neither the Duke modified index [HR=2.0 95% CI (0.65-6.11), p=0.23] nor the REACH [HR=1.5 95% CI (0.2-11.5, p=0.70] or SMART [HR=2.8 95% CI (0.8-10.3), p=0.11] risk scores showed such predictive capabilities. With only 7 myocardial infarctions in 61 patients imaged recently after myocardial infarction, none of the covariates emerged as predictors of myocardial infarction. However, in this population, CMA once again demonstrated the most favorable hazard ratios of the factors assessed (HR=3.1 (95% CI (0.7-15.9) p=0.18) compared to TBR HR=1.5 (95% CI (0.3-6.6) p=0.60), coronary calcium scores HR=0.9 (95% CI (0.2-43.2) p=0.68) and clinical variables (all p>0.50). When patients were divided according to the time from myocardial infarction to PET imaging, we observed no difference in CMA values between those scanned within 13 days from the adverse event and those who underwent PET at least 14 days after infarction (1.39 [0.13-2.70] and 1.19 [0.34-2.87], p=0.38).

Online Tables

Online Table 1. Study participants.

Study	NCT02110303 DIAMOND Dual Antiplatelet Therapy to Reduce Myocardial Injury n=193	NCT01749254 Novel Imaging Approaches to Identify Unstable Coronary Plaques n=78	NCT02607748 ^{18}F-NaF PET for Identifying Coronary Atherosclerotic Plaques n=22	Total (n=293)
Stable angina	n=202(193) (9 excluded as no available baseline PET/CT)	n=40(39) (1 excluded as no available baseline PET/CT)	N/A	232
STEMI/NSTEMI	N/A	n=40 (39) (1 excluded as no available baseline PET/CT)	n= 22	61

Online Table 2. Study exclusion criteria

Study Exclusion criteria	NCT02110303 DIAMOND Dual Antiplatelet Therapy to Reduce Myocardial Injury	NCT01749254 Novel Imaging Approaches to Identify Unstable Coronary Plaques	NCT02607748 ^{18}F-NaF PET for Identifying Coronary Atherosclerotic Plaques
Age	<40	<50	<18
DM	N/A	insulin-dependent diabetes mellitus or pt with BG>11mmol/L were excluded	N/A
Pregnant/breast feeding women	Excluded	Excluded	Excluded
ACS within the last 12 months	Excluded	N/A	N/A
Dual anti-platelet therapy or warfarin or NOAC	Excluded	N/A	N/A
PCI/CABG within the last 3 months (or	Excluded	N/A	N/A

plan for PCI/CABG in the next 12 months)			
Bleeding diathesis	Excluded	N/A	N/A
Renal dysfunction	eGFR<30	Serum creatinine >250 μmol/L	Serum creatinine >132 μmol/L
Known contrast allergy	Excluded	Excluded	Excluded
Inability to provide informed consent	Excluded	Excluded	Excluded
Permanent atrial fibrillation/Multiple premature ventricular or atrial contractions	N/A	N/A	Excluded
EF<35% or Class III congestive heart failure	N/A	N/A	Excluded

Online Table 3. Baseline Characteristics of Study Participants. Comparison of patients with (coronary microcalcification activity [CMA] > 0) and without (CMA = 0) increased coronary ^{18}F-NaF uptake.

	Entire Cohort	CMA=0 (n=90)	CMA>0 (n=203)	
Age in years, mean (SD)	65 (9)	63 (9)	65 (9)	P=0.0803
Men, n (%)	245 (84%)	71 (78.9%)	174 (86.1%)	p=0.1712
Body-mass index (kg/m^2), mean (SD)	29 (5)	29 (5)	29 (5)	P=1.0000
Systolic blood pressure (mm Hg), mean (SD)	141 (20)	137 (19)	144 (20)	**P=0.0054**
Diastolic blood pressure (mm Hg), mean (SD)	79 (11)	78 (9)	80 (12)	P=0.1584
Cardiovascular history, n (%)				
History of ACS	161 (55.1 %)	56 (62.2%)	105 (51.7%)	P=0.0749
History of PCI	237 (80.9 %)	57 (63.3%)	126 (62.1%)	P=0.8963
History of CABG	48 (16.4 %)	16 (17.8%)	32 (15.8%)	P=0.7326
History of angina	136 (46.6 %)	48 (53.3%)	88 (43.43%)	P=0.1283
CVA/TIA	9 (3.1 %)	2 (2.2%)	7 (3.4%)	P=0.7264
Comorbidities/risk factors, n (%)				

Hypertension	174 (59.6 %)	47 (52.2%)	128(63.1%)	P=0.0936
Hyperlipidemia	257 (88 %)	81 (90.0 %)	177 (87.2%)	P=0.1571
Diabetes Mellitus	61 (20.8 %)	16 (17.8%)	35 (22.2%)	P=1.0000
Current smoking	58 (19.9 %)	26 (28.9%)	32 (16.1%)	p=0.0113
Ex-smoker	137 (46.9%)	46 (51.1%)	91 (45.7%)	p=0.3745
Atrial fibrillation	10 (3.4 %)	5 (5.6 %)	5 (2.5%)	P=0.1827
Peripheral vascular disease	16 (5.5 %)	7 (7.8%)	9 (4.4%)	P=0.2698
Medications, n (%) *				
Aspirin	268 (91.8 %)	81 (90.0%)	187 (92.1%)	P=0.8185
P2Y12 antagonist	45 (15.4 %)	12 (13.3%)	33 (16.3%)	P=0.6005
Statin	262 (89.7 %)	80 (86.7%)	183 (90.1%)	P=0.8348
Beta Blocker	196 (67.1 %)	64 (71.1%)	133 (65.5%)	P=0.4184
ACEI/ARB	197 (67.4 %)	64 (71.1%)	134 (66.0%)	P=0.4194
Insulin	4 (1.4 %)	0 (0%)	4 (2.0%)	P=0.3161
Oral diabetic medications	48 (16.4 %)	17 (18.8%)	31 (15.2%)	P=0.4943
Calcium channel blockers	63 (21.6 %)	17 (18.9%)	46 (22.7%)	P=0.5388
Diuretics	38 (16.0 %)	12 (13.3%)	10 (4.9%)	0.0162
Biomarkers, median (IQR)				
Total Cholesterol (mmol/L)	4.1 (3.6-4.7)	4.1 (3.7-4.8)	4.1 (3.5-4.7)	P=0.81034
LDL (mmol/L)	1.9 (1.2-2.4)	1.9 (1.3-2.3)	1.9 (1.2- 2.5)	P=0.93624
HDL (mmol/L)	1.2 (1-1.7)	1.1 (0.9-1.6)	1.3 (1.0-1.7)	P=0.08186
TAG (mmol/L)	1.5 (1.1-2.3)	1.6 (1.1-2.4)	1.5 (1.1-2.3)	P=0.52218

Creatinine (µmol/L)	0.9 (0.8-1.0)	0.9 (0.8-1.0)	0.9 (0.8-1.0)	P=0.80258
CAD, n (%)				
Non-obstructive	15 (5.1 %)	7 (7.8%)	6 (3.0%)	P=0.1190
disease (<50%)	87 (29.8 %)	23 (25.6%)	67 (33.0%)	P=0.2191
- Single-vessel disease	110 (37.7 %)	39 (43.3%)	71 (35.0%)	P=0.1919
- Two-vessel disease	81 (27.6 %)	19 (23.3%)	59 (29.1%)	P=0.1970
- Three-vessel disease	18 (6.1 %)	2 (2.2%)	12 (5.9%)	P=0.2396
- LMS involvement				
Coronary Stent, n (%)	218 (73.4%)	61 (67.8%)	157 (77.3%)	P=0.1099
Segment involvement score. Median (IQR)	5 (3-7)	5 (3-7)	6 (4-7)	P=0.0562
SIS breakdown, n (%)				
0-1	20 (6.8%)	11 (12.2%)	9 (4.4%)	**P=0.0222**
2-3	55 (18.8%)	18 (20.0%)	37 (18.3%)	P=0.7468
4-5	73 (24.9%)	20 (22.2%)	53 (26.1%)	P=0.5587
>5	145 (49.4%)	41 (45.6%)	104 (51.2%)	P=0.3787
Modified Duke CAD index (IQR)	4 (3-5)	4 (3-5)	4 (3-5)	P=0.0574
Coronary Calcium Score, median (IQR)	334 (76-804)	179 (48-529)	378 (103-993)	**P=0.0027**
Coronary Calcium Score, n(%)		36 (40.0%)	48 (23.6%)	
0-99	84 (28.7%)	22 (24.4%)	53 (26.6%)	**P=0.0052**

100-399	76 (25.9%)	23 (25.6%)	52 (25.2%)	P=0.8847
400-999	74 (25.3%)	9 (10.0%)	50 (24.6%)	P=1.0000
>1000	59 (20.1%)			**P=0.0042**
TBR, median (IQR)	1.22 (1.1-1.42)	1.05 (0.99-1.11)	1.29 (1.19-1.48)	**P<0.0001**
TBR>1.28	113 (38.6%)	6 (6.7%)	107 (52.7%)	**P=0.0001**
CMA, median (IQR)	0.66 (0-2.84)	0	0.89 [0.47-3.10]	**P<0.0001**
Coronary Calcium Score>1000	59 (20.1%)	9 (10.0%)	50 (24.6%)	**P=0.0042**
Coronary Calcium Score>1199	46 (15.7%)	9 (10.0%)	37 (18.2%)	P=0.0831
REACH score CV event (IQR)	13 (11-15)	12 (10-14)	13 (11-15)	P=0.08012
SMART risk score % (IQR)	18 (13-26)	17 (13-23)	19 (13-27)	**P=0.0285**
MI during follow-up	20 (6.8%)	0 (0%)	20 (9.9%)	**P=0.0007**
MACE during follow-up	40 (13.6%)	7 (7.8%)	33 (16.3%)	P=0.0643

ACEI/ARB – angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS – acute coronary syndrome, CABG – coronary artery bypass graft, CAD – coronary artery disease, CMA – coronary microcalcification activity, CVA - Cerebrovascular accident, MACE – major adverse cardiovascular event, PCI – percutaneous coronary intervention, REACH - Reduction of Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease, SIS – segment involvement score, TAG - triacylglycerides, TBR – target to background ratio, TIA - transient ischemic attack

*medications at the time of scan

Online Table 4. Comparison of patient with and without myocardial infarction (MI) or coronary heart disease death during follow-up.

	Fatal or non-fatal myocardial infarction (n=20)	Fatal or non-fatal myocardial infarction (n=273)	
Age in years, mean (SD)	64 (9)	65 (9)	P=0.6318
Men, n (%)	18 (90%)	227 (83.5%)	P=0.5465
Body-mass index (kg/m^2), mean (SD)	30 (5)	29 (5)	P=0.3886
Systolic blood pressure (mm Hg), mean (SD)	144 (23)	141 (20)	P=0.5222
Diastolic blood pressure (mm Hg), mean (SD)	78 (14)	79 (11)	P=0.7007
Cardiovascular history, n (%)			
H of ACS	9 (45%)	153 (55.4%)	P=0.3603
H of PCI	17 (85%)	220 (80.6%)	P=0.4821
H of CABG	5 (25%)	43 (15.6%)	P=0.3424
H of angina	13 (65%)	123 (44.6%)	P=0.1049
CVA/TIA	2 (10%)	7 (2.5%)	P=0.1187
Comorbidities/risk factors, n (%)			
HTN	17 (85%)	158 (57.9%)	**P=0.0179**
HPL	19 (95%)	239 (87.5%)	P=0.4859

DM	6 (30%)	55 (20.2%)	P=0.3893
Current smoking	4 (20%)	54 (20%)	P=1.0000
Ex-smoker	9 (45%)	128 (47.4%)	P=1.0000
Atrial fibrillation	1 (5%)	9 (3.3%)	P=0.5125
Peripheral vascular disease	2 (10%)	14 (4.1%)	P=0.2994
Medications, n (%) *			
Aspirin	18 (90%)	251 (91.9%)	P=0.6729
P2Y12 antagonist	11 (55%)	34 (12.5%)	**P<0.0001**
Statin	19 (95%)	244 (89.4)	P=0.7050
Beta Blocker	14 (70%)	183 (67%)	P=1.0000
ACEI/ARB	13 (65%)	152 (55.7%)	P=0.4889
Insulin	0 (0%)	4 (1.5%)	P=1.0000
Oral diabetic medications	6 (30%)	42 (15.4%)	P=0.1123
CCB	7 (35%)	56 (20.5%)	P=0.1571
Diuretics	3 (15%)	33 (12.1%)	P=0.7220
Biomarkers, median (IQR)			
Total Cholesterol (mmol/L)	4 (3.0-4.6)	4.1 (3.6–4.8)	P=0.4354
LDL (mmol/L)	1.4 (0.9-2.8)	1.9 (1.3-2.4)	P=0.1443
HDL (mmol/L)	1.3 (1.0-2.0)	1.2 (1.0-1.7)	P=0.54186

TAG (mmol/L)	2.0 (1.1-2.3)	1.5 (1.1 -2.3)	P=0.4593
Creatinine (µmol/L)	78 (69-88)	78 (70 – 91)	P=0.85716
CAD, n (%)			
- Non-obstructive disease (<50%)	0	15 (5.5%)	P=0.6097
- Single vessel disease	6 (30.0%)	82 (30%)	P=1.0000
- Two vessel disease	7 (35.0%)	103 (37.7%)	P=1.0000
- Three vessel disease	7 (35.0%)	73 (26.7%)	P=0.4400
- LMS involvement	3 (15.0%)	15 (5.5%)	P=0.1145
Coronary Stent, n (%)	15 (75%)	203 (74.4%)	P=1.0000
Segment involvement score. Median (IQR)	6 (4-8)	5 (3-7)	P=0.25014
SIS breakdown, n (%)			
0-1	1 (5%)	19 (7.0%)	P=1.0000
2-3	2 (10%)	53 (19.4%)	P=0.3868
4-5	7 (35%)	66 (24.1%)	P=0.2894
>5	10 (50%)	135 (49%)	P=1.0000
Duke index	5 (4-5)	4 (3-5)	P=0.0536
Coronary Calcium Score, median (IQR)	397 (39-1456)	331 (76-775)	P=0.60306

Coronary Calcium Score, n (%)			
0-99	6 (30%)	78 (28.6%)	P=1.0000
100-399	4 (20%)	99 (36.3%)	P=0.2238
400-999	4 (20%)	71 (26.1%)	P=0.7909
>1000	6 (30%)	25 (9.0%)	**P=0.0114**
TBR, median (IQR)	1.40 (1.28-1.77)	1.21 (1.09-1.40)	**P=0.0008**
CMA, median (IQR)	3.05 (1.62-5.25)	0.46 (0-2.47)	**P=0.00012**
TBR>1.275	16 (80%)	97 (36%)	**P=0.0002**
CMA>0	20 (100%)	183 (67%)	**P=0.0007**
CMA>1.56	16 (80%)	93 (34.1%)	**P=0.0001**
Coronary Calcium Score>1000	7 (35%)	52 (19.0%)	**P=0.0001**
REACH score CV event (IQR)	13 (11-15)	13 (11-15)	P=0.79486
SMART risk score	20 (13-28)	18 (13-26)	P=0.5157

ACEI/ARB – angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS –

acute coronary syndrome, CABG – coronary artery bypass graft, CAD – coronary artery disease,

CMA – coronary microcalcification activity, CVA - Cerebrovascular accident, MACE – major

adverse cardiovascular event, PCI – percutaneous coronary intervention, REACH - Reduction of

Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease,

SIS – segment involvement score, TAG - triacylglycerides, TBR – target to background ratio,

TIA - transient ischemic attack

*medications at the time of scan

Online Table 5. Comparison of patients scanned shortly after myocardial infarction (unstable) and those who had stable coronary artery disease at the time of imaging.

	Stable (n=232)	Unstable (n=61)	
Age in years, mean (SD)	66 (8)	62 (9)	**P=0.0008**
Men, n (%)	188 (81%)	57 (93.4%)	P=0.0288
Body-mass index (kg/m^2), mean (SD)	30 (5)	28 (5)	P=0.0058
Systolic blood pressure (mm Hg), mean (SD)	145 (19)	130 (19)	**P<0.0001**
Diastolic blood pressure (mm Hg), mean (SD)	80 (11)	76 (13)	**P=0.0157**
Cardiovascular history, n (%)			
H of ACS	153 (65.9 %)	9 (14.8%)	**P<0.0001**
H of PCI	176 (75.9 %)	7 (11.5%)	**P<0.0001**
H of CABG	46 (19.8 %)	2 (3.3%)	**P=0.0008**
H of angina	127 (54.7 %)	9 (14.8%)	**P<0.0001**
CVA/TIA	6 (2.6 %)	3 (4.8%)	P=0.4004
Comorbidities/risk factors, n (%)			
HTN	143 (61.6 %)	32 (52.5%)	P=0.2404
HPL	224 (96.6 %)	34 (55.7%)	**P<0.0001**
DM	49 (21.1 %)	12 (19.7%)	P=0.8613

Current smoking	33 (14.2 %)	25 (43.9%)	**P<0.0001**
Ex-smoker	117 (50.4 %)	20 (30.8%)	**P=0.0147**
Atrial fibrillation	6 (2.6 %)	4 (6.6%)	P=0.2250
Peripheral vascular disease	14 (6.0 %)	2 (3.3%)	P=0.5375
Medications, n (%)*			
Aspirin	221 (95.3 %)	48 (78.7%)	**P=0.0002**
PY12 antagonist	11 (4.7 %)	34 (55.7%)	**P<0.0001**
Statin	217 (93.5 %)	46 (75.4%)	**P=0.0002**
Beta Blocker	160 (69.0 %)	37 (60.7%)	P=0.2238
ACEI/ARB	169 (72.9 %)	29 (47.5%)	**P=0.0003**
Insulin	3 (1.3 %)	1 (1.6%)	P=1.0000
Oral diabetic medications	43 (18.5 %)	5 (8.2%)	P=0.0537
CCB	56 (24.1 %)	7 (11.5%)	**P=0.0354**
Diuretics	29 (12.5 %)	9 (14.8%)	**P<0.0001**
Biomarkers, median (IQR)			
Total Cholesterol (mmol/L)	4.0 (3.6-4.7)	4.4 (3.5-5.0)	**P<0.04884**
LDL (mmol/L)	2.1 (1.5-2.5)	1.5 (0.8)	**P<0.0001**
HDL (mmol/L)	1.2 (1.0-1.5)	2.2 (1.2)	**P<0.0001**
TAG (mmol/L)	1.5 (1.1-2.2)	2.2 (1.5)	**P=0.02088**
Creatinine (μmol/L)	77 (70-89)	86 (24)	P=0.31732
CAD, n (%)			

Non-obstructive disease (<50%)	14 (6%)	1 (1.6%)	P=0.3228
- **Single vessel disease**	60 (25.9 %)	28 (45.9%)	**P=0.0044**
- **Two vessel disease**	95 (40.9 %)	15 (24.6%)	**P=0.0252**
- Three vessel disease	63 (27.2 %)	17 (27.8%)	P=1.0000
- LMS involvement	15 (5.2 %)	3 (4.9%)	P=1.0000
Coronary Stent, n (%) Anywhere	168 (72.4 %)	50 (80.6%)	P=0.2440
Segment involvement score. Median (IQR)	6 (4-7)	4 (2-7)	**P=.00438**
SIS breakdown, n (%)			
0-1	13 (5.6%)	7 (11.5%)	P=0.1483
2-3	36 (15.5%)	19 (31.1%)	**P=0.0091**
4-5	57 (24.6%)	16 (26.3%)	P=0.8680
>5	126 (54.3%)	19 (31.1%)	**P=0.0015**
CCS, median (IQR)	416 (104-937)	106 (7-355)	**P<0.00001**
CCS, n (%)			
0-99	84 (28.7%)	29 (47.5%)	P=0.1389
100-399	76 (25.9%)	18 (29.5%)	P=0.7581
400-999	74 (25.3%)	9 (14.8%)	**P=0.0101**
>1000	54 (23.3%)	5 (8.2%)	**P=0.0072**
TBR, median (IQR)	1.21 (1.1- 1.4)	1.23 (1.15-1.45)	P= 0.15272
CMA, median (IQR)	0.40 (0-2.75)	1.23 (0.22-3.00)	P=0.03

CMA>0	151 (65%)	52 (85%)	**P=0.0028**
CMA>1.56	82 (35.3%)	27 (52.6%)	P=0.2340
TBR>1.275	84 (36,1%)	29 (47.5%)	P=0.1389
CCCS>1000	54 (23.3%)	5 (8.2%)	**P=0.0045**
CCCS>1199	42 (18.1%)	3 (4.9%)	**P=0.0091**
REACH score (IQR) CV event	13 (11-15)	12 (11-15)	P=0.06724
20-month risk of next CV event, % (IQR)	6.3 (4.7-8.5)	5.4 (4.4-7.9)	P=0.08364
REACH score (IQR) CV death	11 (10-13)	11 (9-13)	P=0.09492
20-month cardiovascular death, % (IQR)	1.8 (1.4-3.0)	1.8 (1.1-2.7)	P=0.11184
Duke score	4 (3-5)	4 (3-5)	P=0.2202
SMART risk score (%)	20 (14-27)	13 (10-20)	**P=0.00014**
Infarct during follow-up	13 (6%)	7 (11%)	P=0.18

ACEI/ARB – angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS –

acute coronary syndrome, CABG – coronary artery bypass graft, CAD – coronary artery disease,

CMA – coronary microcalcification activity, CVA - Cerebrovascular accident, MACE – major

adverse cardiovascular event, PCI – percutaneous coronary intervention, REACH - Reduction of

Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease,

SIS – segment involvement score, TAG - triacylglycerides, TBR – target to background ratio,

TIA - transient ischemic attack

*medications at the time of scan

Online Table 6. Comparison of patients with and without major adverse cardiovascular events during follow-up.

	MACE (n= 40)	No MACE (n=253)	
Age in years, mean (SD)	64 (9)	65 (9)	P=0.5143
Men, n (%)	36 (90%)	209 (82.9%)	P=0.3567
Body-mass index (kg/m^2), mean (SD)	29 (5)	29 (5)	P=1.0000
Systolic blood pressure (mm Hg), mean (SD)	138 (21)	142 (20)	P=0.2440
Diastolic blood pressure (mm Hg), mean (SD)	76 (13)	79 (11)	P=0.1194
Cardiovascular history, n (%)			
H of ACS	16 (40.0%)	146 (57.7%)	**P=0.0409**
H of PCI	36 (90.0%)	201 (79.4%)	P=0.2836
H of CABG	6 (15.0%)	42 (16.6%)	P=1.0000
H of angina	24 (60.0%)	112 (44.3%)	P=0.1246
CVA/TIA	4 (10.0%)	5 (2.0%)	**P=0.0227**
Comorbidities/risk factors, n (%)			
HTN	27 (67.5%)	148 (58.5%)	P=0.3033
HPL	34 (85.0 %)	224 (88.5%)	P=0.5981

DM	11 (27.5 %)	50 (19.8%)	P=0.2947
Current smoking	7 (17.5 %)	51 (20.4%)	P=0.8322
Ex-smoker	17 (42.5 %)	120 (48.0%)	P=0.6115
Atrial fibrillation	2 (5.0 %)	8 (3.2%)	P=0.6315
Peripheral vascular disease	6 (15.0%)	10 (4.0%)	**P=0.0126**
Medications, n (%) *			
Aspirin	36 (90.0%)	233 (92.1%)	P=0.7547
P2Y12 antagonist	18 (45.0%)	27 (10.7%)	**P<0.0001**
Statin	37 (92.5%)	226 (89.3%)	P=0.7792
Beta Blocker	26 (65.0%)	171 (67.6%)	P=0.7213
ACEI/ARB	23 (57.5%)	175 (69.1%)	P=0.0924
Insulin	1 (2.5%)	3 (1.2%)	P=0.4459
Oral diabetic medications	8 (20.0%)	40 (15.9%)	P=0.4944
CCB	13 (32.5%)	50 (19.8%)	P=0.0953
Diuretics	8 (20.0%)	30 (11.9%)	0=0.2012
Biomarkers, median (IQR)			
Total Cholesterol (mmol/L)	4.0 (3.4-4.6)	4.1 (3.6-4.8)	P=0.39532
LDL (mmol/L)	1.6 (0.9-2.3)	2.0 (1.4-2.5)	**P=0.0455**
HDL (mmol/L)	1.6 (1.0-2.0)	1.2 (1.0-1.7)	P=0.06432
TAG (mmol/L)	1.5 (1.1-2.3)	1.5 (1.1-2.3)	P=0.85716
Creatinine (μmol/L)	78 (69-94)	78 (70-90)	P=0.57548
CAD, n (%)			

Non-obstructive disease (<50%)	2 (5.0%)	13 (5.1%)	P=1.0000
- Single-vessel disease	14 (35.0%)	74 (29.2%)	P=0.4625
- Two-vessel disease	13 (32.5%)	97 (38.3%)	P=0.5986
- Three-vessel disease	11 (27.5%)	69 (27.2%)	P=1.000
- LMS involvement	4 (10.0%)	14 (5.5%)	P=0.2642
Coronary Stent, n (%)	29 (72.5%)	189 (74.7%)	P=0.8455
Segment involvement score. Median (IQR)	5 (4-8)	6 (3-7)	P=0.5485
SIS breakdown, n (%)			
0-1	1 (2.5%)	19 (7.5%)	P=0.3301
2-3	8 (20.0%)	47 (18.6%)	P=0.8286
4-5	14 (35.0%)	59 (23.3%)	P=0.1189
>5	17 (42.5%)	128 (50.6%)	P=0.4902
Duke index	4 (4-5)	4 (3-5)	P=0.16452
Coronary Calcium Score, median (IQR)	195 (50-1126)	344 (81-801)	P=0.4965
Coronary Calcium Score, n (%)			
0-99	14 (35.0%)	70 (27.7%)	P=0.3507
100-399	10 (25.0%)	66 (26.1%)	P=1.0000
400-999	6 (15.0%)	68 (26.8%)	P=0.1207

>1000	10 (25.0%)	49 (19.4%)	P=0.3999
TBR, median (IQR)	1.34 (1.13-1.54)	1.22 (1.10-1.40)	P=0.07346
CMA, median (IQR)	1.9 (1.65-4.76)	0.51 (0-2.42)	**P=0.00988**
TBR>1.28	23 (57.5%)	90 (35.6%)	**P=0.0135**
CMA>0	20 (50%)	170 (67%)	**P=0.0488**
CMA>1.56	23 (57.5%)	86 (34%)	**P=0.0076**
Coronary Calcium Score>1000	10 (25%)	49 (19.4%)	P=0.4012
REACH score CV event (IQR)	13 (11-15)	13 (11-15)	P=0.6672
SMART risk score %	19 (12-28)	18 (13-26)	P=0.76418

ACEI/ARB – angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS –

acute coronary syndrome, CABG – coronary artery bypass graft, CAD – coronary artery disease,

CMA – coronary microcalcification activity, CVA - Cerebrovascular accident, MACE – major

adverse cardiovascular event, PCI – percutaneous coronary intervention, REACH - Reduction of

Atherothrombosis for Continued Health, SMART - Secondary Manifestations of Arterial

Disease, SIS – segment involvement score, TAG - triacylglycerides, TBR – target to background

ratio, TIA - transient ischemic attack

Online Table 7. Uni- and multivariable Cox proportional regression models for prediction of major adverse cardiovascular events during follow-up.

	Coronary Microcalcification Activity >1.56		Target to background ratio >1.275		Coronary Calcium Score > 1199	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Model 1	2.29 (1.22-4.29)	0.01	2.08 (1.11-3.89)	0.02	1.94 (0.94-3.96)	0.071
Model 2	2.36 (1.23-4.52)	0.01	2.09 (1.09-4.01)	0.027	-	
Model 3	2.19 (1.15-4.17)	0.017	2.06 (1.08-3.96)	0.029	1.93 (0.85-4.38)	0.115
Model 4	2.08 (1.06-4.07)	0.033	1.79 (0.91-3.55)	0.093	1.68 (0.73-3.84)	0.221
Model 5	2.46 (1.25-4.83)	0.009	2.06 (1.04-4.07)	0.038	-	
Model 6	2.10 (1.07-4.13)	0.030	1.80 (0.90-3.61)	0.098		

Model 1 – unadjusted; Model 2 – adjusted for Coronary Calcium Score; Model 3 – adjusted for segment involvement score, number of coronary stents, multivessel coronary artery disease; Model 4 – adjusted for segment involvement score, number of coronary stents, multivessel coronary artery disease, age, gender, hyperlipidemia, hypertension, diabetes, smoking; Model 5 – similar to Model 4 and additionally adjusted for coronary calcium scoring. Model 6 - similar to Model 5 and additionally adjusted for REACH and SMART risk scores.

Online Table 8. Prediction of myocardial infarction and major adverse cardiovascular events (MACE) during follow-up. Area under the receiver-operator curve.

	Myocardial Infarction		MACE	
	Area under receiver operator curve (95% CI)	p-value	Area under receiver operator curve (95% CI)	p-value
CMA	0.76 (0.67-0.84)	<0.001	0.61 (0.52-0.71)	0.02
TBR	0.72 (0.62-0.83)	0.001	0.59 (0.49-0.69)	0.07
Coronary Calcium Score	0.55 (0.38-0.69)	0.45	0.48 (0.38-0.59)	0.73
REACH	0.52 (0.38-0.66)	0.77	0.48 (0.38-0.58)	0.70
SMART	0.54 (0.41-0.68)	0.47	0.52 (0.42-0.62)	0.70
Duke index	0.62 (0.49-0.74)	0.08	0.58 (0.48-0.67)	0.12
Age	0.48 (0.35-0.60)	0.71	0.51 (0.38-0.64)	0.52

CMA – coronary microcalcification activity, TBR – target to background ratio, REACH - Reduction of Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease

Online Table 9. Prediction of myocardial infarction and major adverse cardiovascular events (MACE) during follow-up. Univariable Cox proportional regression models with covariates as continuous variables.

	Myocardial Infarction		MACE	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
CMA	1.07 (1.03-1.12)	0.001	1.06 (1.01-1.10)	0.012
TBR	2.57 (1.66-3.98)	<0.001	2.08 (1.35-3.22)	0.001
Coronary Calcium Score	1.00 (1.00-1.01)	0.25	1.00 (1.00-1.00)	0.77
REACH	1.00 (0.86-1.16)	0.99	0.96 (0.86-1.07)	0.96
SMART	1.02 (0.98-1.06)	0.26	1.02 (0.99-1.05)	0.13
Duke index	1.59 (0.99-2.48)	0.07	1.23 (0.94-1.62)	0.14
Age	0.99 (0.94-1.04)	0.61	0.99 (0.95-1.03)	0.55

CMA – coronary microcalcification activity, TBR – target to background ratio, REACH - , CI-Confidence interval, Reduction of Atherothrombosis for Continued Health, SMART - Secondary Manifestations of Arterial Disease

Online Table 10. Prediction of myocardial infarction and major adverse cardiovascular events (MACE) during follow-up by age and risk scores.

	Age > 53.5		REACH risk score > 17.5		SMART risk score > 18.5		Duke CAD index > 4.5	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Myocardial Infarction	1.22 (0.28-5.27)	0.78	2.11 (0.49-9.10)	0.32	2.05 (0.82-5.13)	0.13	1.80 (0.75-4.36)	0.19
MACE	0.54 (0.25-1.18)	0.12	1.71 (0.53-5.54)	0.38	1.47 (0.78-2.76)	0.23	1.22 (0.66-2.28)	0.53

CAD – Coronary Artery Disease, MACE – Major Adverse Cardiovascular Event, REACH - Reduction of Atherothrombosis for Continued Health, SMART Secondary Manifestations of Arterial Disease

Online Figures

Online Figure 1. Measuring Disease Activity Across the Coronary Vasculature with ^{18}F-NaF Coronary Microcalcification Activity (CMA). 3-Dimensional rendering of coronary CT angiography with superimposed tubular whole vessel volumes of interest (light green) employed for evaluation of ^{18}F-sodium fluoride uptake (blue and red). Compared to target to background ratio (TBR) measurements (which depend on single pixel tracer uptake), the coronary microcalcification activity (CMA) is a summary measure of ^{18}F-NaF activity across the entire coronary vasculature as it includes all counts originating from the coronary arteries (uptake exceeding the threshold of background blood pool activity + 2 standard deviations).
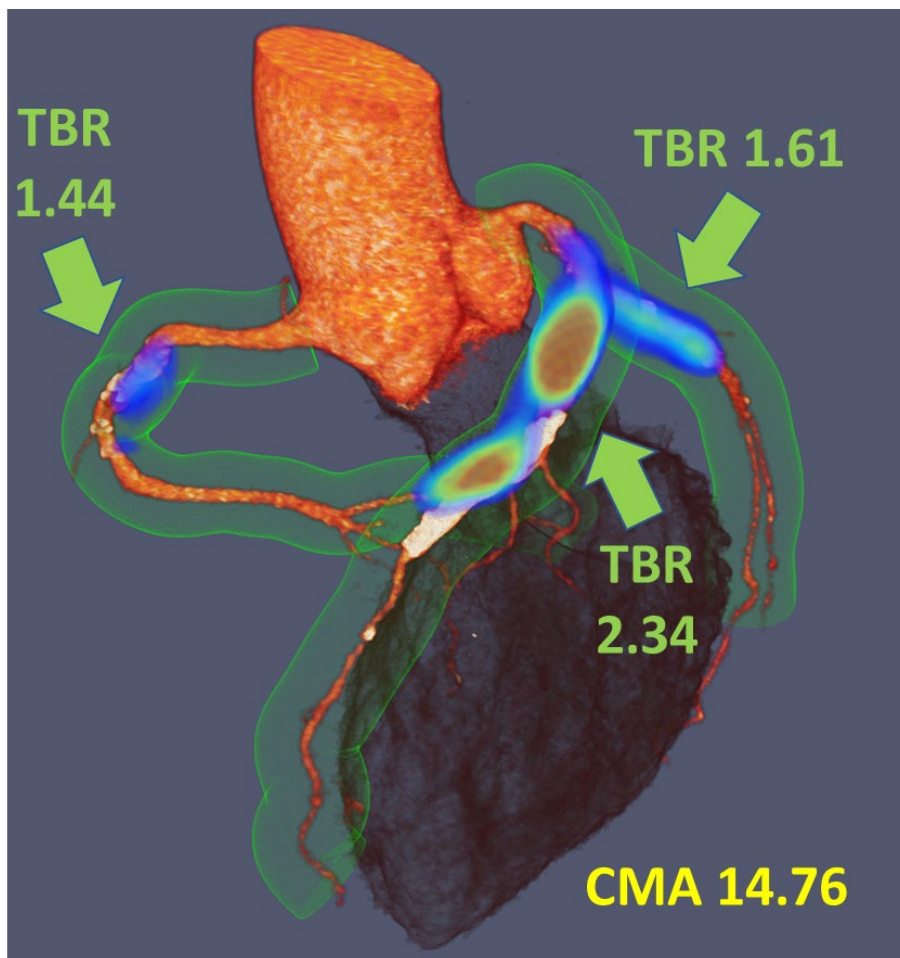
Online Figure 2. Interobserver Reproducibility of the coronary microcalcification activity (CMA).

Interobserver reproducibility of CMA measurements

ICC 0.992 (95% CI 0.980-0.998)
p<0.001
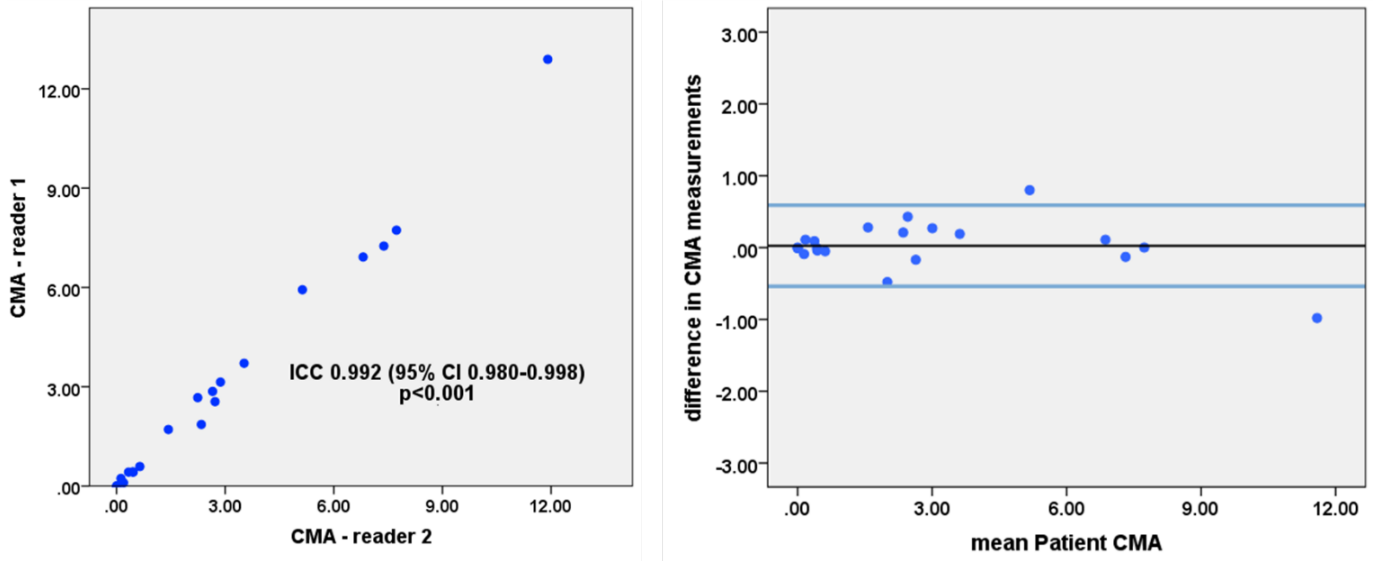
Online Figure 3. Receiver Operator Curve Analysis for the prediction of myocardial infarction in the study population (A), in patients with established stable coronary artery disease (B) and imaged shortly after myocardial infarction (C). Receiver operator curves for myocardial infarction prediction by coronary ^{18}F-NaF PET (CMA), CT coronary calcium score, modified Duke index on CTCA and the REACH clinical risk scores.
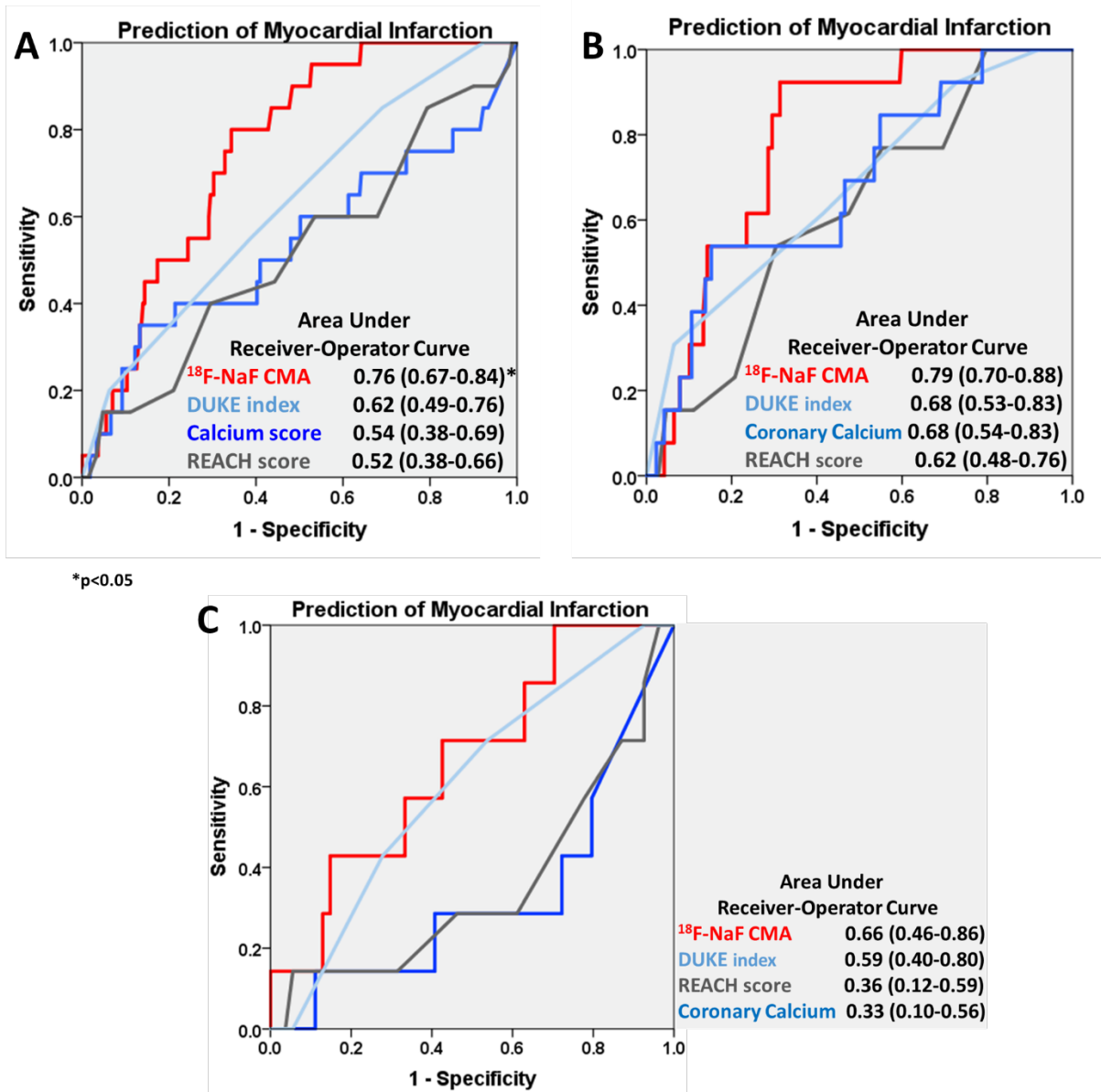
Online Figure 4. Coronary disease activity and plaque burden in patients with and without future major adverse cardiovascular events. Coronary microcalcification activity (CMA, top row), maximum target to background ratios (TBR, middle row) and the coronary calcium scores (CCS, bottom row) in patients with and without MACE during follow-up. For the Kaplan-Meier curves patients were dichotomized according to thresholds derived from receiver operator curves using the Youden's index: CMA=1.56, TBR=1.28 and coronary calcium score = 1199 Agatston-units.

Coronary Microcalcification activity (CMA)

P=0.008

High CMA

Low CMA

No. at risk
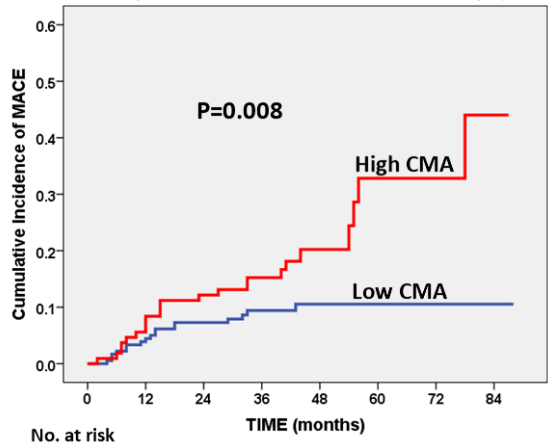
| 109 | 97 | 95 | 86 | 25 | 14 | 5 | 3 |
| 184 | 168 | 163 | 101 | 51 | 35 | 12 | 8 |

Target to Background Ratio (TBR)

P=0.019

High TBR

Low TBR

No. at risk
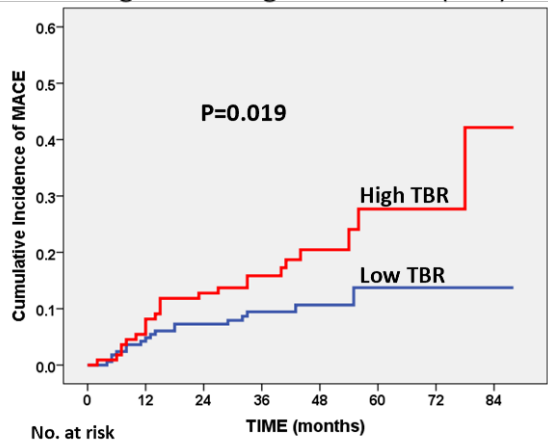
| 113 | 100 | 97 | 82 | 27 | 15 | 6 | 3 |
| 180 | 165 | 161 | 105 | 49 | 34 | 11 | 8 |

Coronary Calcium Score (CCS)

High CCS

p=0.07

Low CCS

No. at risk
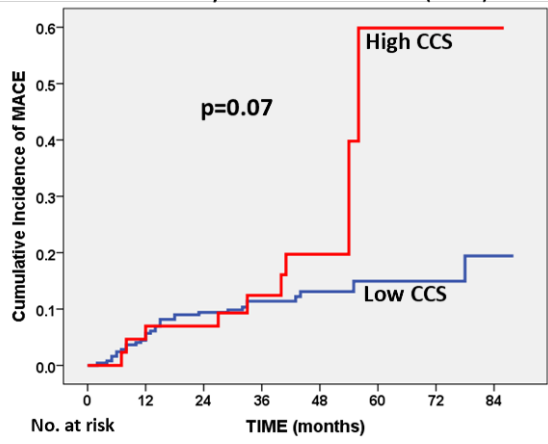
| 45 | 40 | 36 | 27 | 18 | 7 | 4 | 2 |
| 248 | 225 | 222 | 160 | 58 | 42 | 13 | 12 |

34

Online Figure 5. Kaplan Meier Curve Analysis for the prediction of myocardial infarction in patients imaged shortly after myocardial infarction (A) and in patients with established stable coronary artery disease (B).

Coronary Microcalcification activity (CMA)
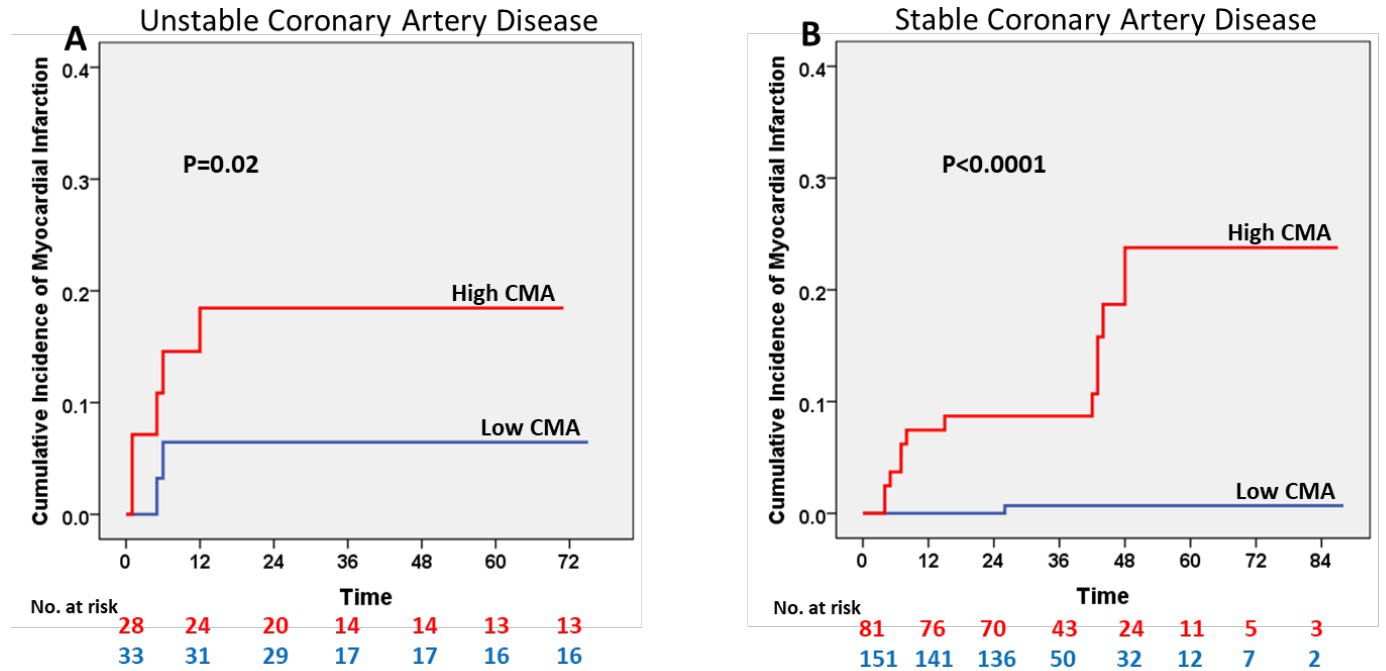
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Patients with advanced established coronary artery disease

Coronary disease activity on ^{18}F-NaF PET: the only predictor of fatal or non-fatal MI
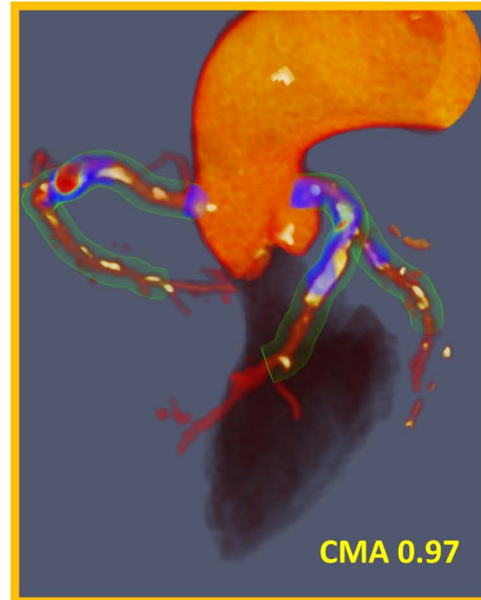
(independent of calcium score, coronary artery lumen stenosis, risk scores & co-morbidities)

CMA 0.00
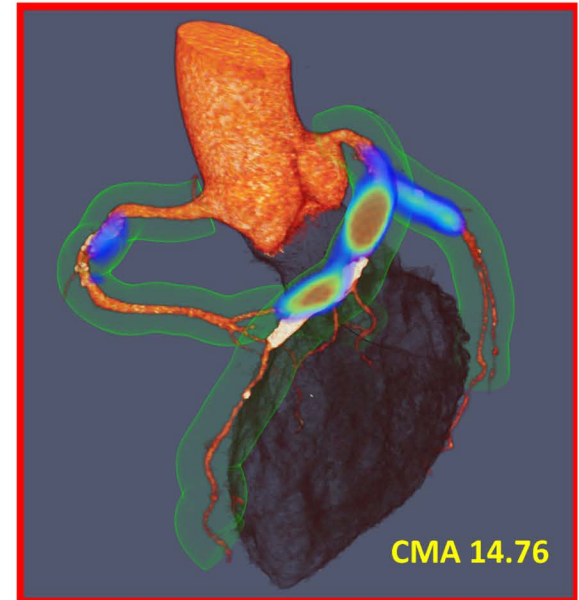
CMA 0.97

CMA 14.76
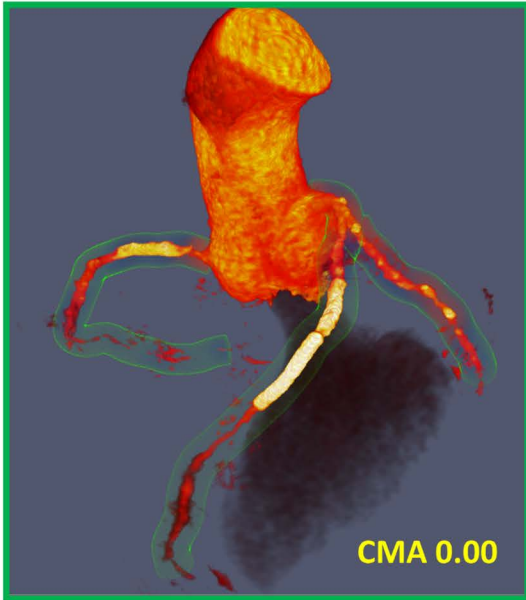
No Activity
(CMA=0)
No MI on follow up
Continue medical therapy

Low Activity
(CMA 0.01 to 1.56)
Intermediate risk
Close Observation

High Activity
(CMA > 1.56)
8-fold risk of future MI
Intensify Therapy

Central Illustration