In vivo detection of high-risk coronary plaques by radiofrequency intravascular ultrasound and cardiovascular outcome

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ABSTRACT

Aims: Acute coronary syndromes (ACS) are mostly caused by plaque rupture. This study aims to investigate the prognostic value of *in-vivo* detection of high risk coronary plaques by intravascular ultrasound (IVUS) in patients undergoing coronary angiography.

Methods and results: Between November 2008 and January 2011, IVUS of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for ACS (n=318) or stable angina (n=263). Primary endpoint was major adverse cardiac events (MACE), defined as mortality, ACS or unplanned coronary revascularization. Culprit lesion-related events were not counted. Cumulative Kaplan-Meier incidence of 1-year MACE was 7.8%. The presence of IVUS virtual histology-derived thin-cap fibroatheroma (TCFA) lesions (present 10.8% vs. absent 5.6%; adjusted HR 1.98, 95%CI 1.09-3.60; p=0.026) and lesions with a plaque burden of ≥70% (present 16.2% vs. absent 5.5%; adjusted HR 2.90, 95%CI 1.60-5.25; p<0.001) were independently associated with higher MACE rate. TCFA lesions were also independently associated with the composite of death or ACS only (present 7.5% vs. absent 3.0%; adjusted HR 2.51, 95%CI 1.15-5.49; p=0.021). TCFA lesions with a plaque burden of ≥70% were associated with higher MACE rate within (p=0.011) and after (p<0.001) 6 months of follow-up, while smaller TCFA lesions were only associated with higher MACE rate after 6 months (p=0.033).

Conclusion: In patients undergoing coronary angiography, the presence of IVUS virtual histology-derived TCFA lesions in a non-culprit coronary artery is strongly and independently predictive for occurrence of MACE within 1 year, particularly of death and ACS. TCFA lesions with a large plaque burden carry higher risk than small TCFA lesions, especially on the short term.

INTRODUCTION

Acute coronary syndromes (ACS) are expected to remain the leading cause of mortality and morbidity in the upcoming years.(1) Patients with a history of cardiovascular disease have an increased risk for ACS.(2) Post-mortem studies have shown that ACS is mostly caused by thin-cap fibroatheroma (TCFA) lesions.(3-5) Detection of these coronary lesions that are at high risk to rupture may be highly relevant for further improvement of prognostication and for optimal choice of treatment. However, these high risk lesions cannot be easily detected by coronary angiography.(6)

Intravascular ultrasound (IVUS) radiofrequency analyses, also known as IVUS virtual histology, allows for differentiation of various plaque phenotypes and may therefore be well suited for detection of plaques that are at high risk to rupture.(7-9) The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study has shown that plaque characteristics as assessed by IVUS were independently predictive for recurrent cardiac events in patients admitted with an ACS.(10) However, the events in PROSPECT were mainly driven by rehospitalizations for unstable or progressive angina, while less is known about the prognostic value of IVUS for acute cardiac events as a consequence of spontaneous plaque rupture (i.e. recurrent ACS or death). Furthermore, the prognostic value of IVUS in patients with stable angina remains unclear. This study aims to investigate the prognostic value of *in-vivo* detection of high risk plaques by IVUS in patients undergoing coronary angiography for ACS or stable angina.

METHODS

Study population

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described elsewhere.(11) In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for ACS or stable angina pectoris have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. Although this original ATHEROREMO-IVUS cohort was further enriched with eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib versus placebo, these additional IBIS-2 patients were not included in the present analysis in order to prevent possible treatment interaction from darapladib.(12)

The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients. This study is registered with ClinicalTrials.gov, number NCT01789411.

Intravascular ultrasound imaging

Following the standard coronary angiography procedure, IVUS imaging of a non-culprit coronary artery was performed. Selection of the non-culprit vessel was predefined in the study protocol. The order of preference for selection of the non-culprit vessel was: 1. left anterior descending (LAD) artery; 2. right coronary artery (RCA); 3. left circumflex (LCX) artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second. The baseline IVUS images were sent to an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for baseline patient characteristics and clinical outcomes data. The IVUS gray-scale and virtual histology analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. The external elastic membrane and luminal borders were contoured for each frame of the virtual histology-derived dataset. Extent and phenotype of the atherosclerotic plaque were assessed. Plaque burden was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Using IVUS virtual histology, the composition of the atherosclerotic lesions was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.(7) Confluency of the necrotic core and dense calcium, as well as the contact of the necrotic core with the lumen were independently assessed by visual examination, which was performed independently by three investigators (HMG, SPB and JHH) who were blinded to the clinical outcomes. Consensus was reached in case of disagreement. The lesions were further classified into: 1. adaptive intimal thickening (intimal thickening of <600 μm for <20% of the circumference); 2. pathological intimal thickening (intimal thickening ≥600 μm for >20% of the circumference with >15% fibrofatty tissue and no confluent necrotic core or dense-calcium); 3. fibrotic plaque (consisting predominantly of fibrous tissue without confluent necrotic core or dense-calcium); 4. fibrocalcific plaque (presence of >10% confluent dense-calcium without confluent necrotic core); 5. fibroatheroma (presence of >10% confluent necrotic core with an overlying layer of fibrous tissue); 6. calcified fibroatheroma (fibroatheroma containing >10% confluent dense-calcium); 7. non-calcified TCFA (presence of >10% confluent necrotic core in direct contact with the lumen); 8. calcified TCFA (TCFA containing >10% of confluent dense-calcium) (Figure 1).(8) All of the above mentioned criteria should be present in three consecutive frames for a lesion to be considered of a particular category. TCFA lesions with a plaque burden of at least 70% were classified as large TCFA lesions.

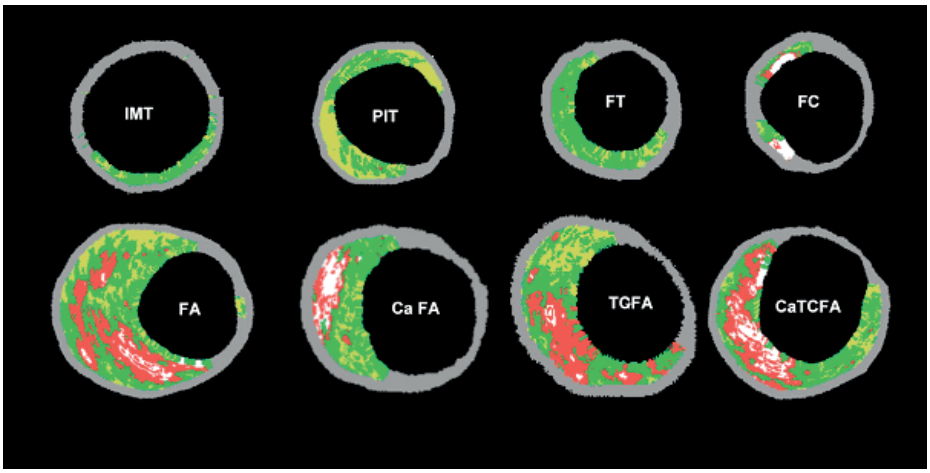


Figure 1. Classification of plaque morphology with intravascular ultrasound virtual histology
IMT, intimal medial thickening; PIT, pathological intimal thickening; FT, fibrotic plaque; FC, fibrocalcific plaque; FA, fibroatheroma; CaFA, calcified fibroatheroma; TCFA, thin-cap fibroatheroma; CaTCFA, calcified thin-cap fibroatheroma.

Study endpoints

Clinical follow-up started at inclusion and lasted 1 year. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed during follow-up. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information whenever necessary. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology.(13) Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). All events were adjudicated as related to a coronary site that was treated during the index procedure (culprit lesion related event) or as related to a coronary site that was not treated during the index procedure (non-culprit lesion related event). Events that were related to both the culprit lesion and a non-culprit site (e.g. revascularization of multiple vessels with CABG) were classified into both categories. When information was not sufficient to classify an event as either culprit lesion related or non-culprit lesion related, the event was classified as indeterminate.

The primary endpoint was MACE, defined as non-culprit lesion related or indeterminate mortality, ACS or unplanned coronary revascularization. The secondary endpoint was defined as the composite of non-culprit lesion related or indeterminate mortality or ACS. Definite culprit lesion related events were not counted in the primary and

secondary endpoint. Occurrence of culprit lesions related events are most probably caused by in-stent restenosis or in-stent thrombosis, while we were only interested in unanticipated, spontaneous MACE. The endpoints were adjudicated by a clinical event committee that had no knowledge of the IVUS data.

Statistical analysis

Under the previously described assumptions (design paper) that high risk lesions (e.g. TCFA) will be present in 30% of the patients and that MACE will occur in 10% of the total study population, our sample size of 581 patients would provide 85% to 99% power to detect a hazard ratio in the range of 2.0 to 2.5 with a two-sided alpha of 0.05.(11)

Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are presented as median and inter-quartile range (IQR). Categorical variables are presented in numbers and percentages. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Cumulative event rates were estimated according to the Kaplan-Meier method. Cumulative Kaplan-Meier event curves were compared by the log-rank test. Cox proportional hazards regression analyses were performed to evaluate the associations between IVUS characteristics and study endpoints. In multivariable analyses, the variables age, gender, diabetes mellitus, hypertension, history of PCI and indication for coronary angiography were considered as potential confounders and were entered into the full model. These covariates (except for indication for coronary angiography) were chosen based on the multivariable model that was used in the PROSPECT study, taking into account the number of events available.(10) The final results are presented as hazard ratios (HR) with 95% confidence interval (95% CI). Z-test for heterogeneity was performed to test for heterogeneity in effect estimates between patients admitted with and without ACS. All statistical analyses were performed at patient level. All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Mean age of the study population was 61.6 ± 11.3 years, 75.6% were men and 17.0% had diabetes mellitus (Table 1). Coronary angiography or PCI was performed for various indications: 28.7% of the patients had an acute myocardial infarction (STEMI and non-STEMI), 26.0% of the patients had unstable angina pectoris and 43.7% of patients had stable angina pectoris. Median length of the imaged coronary segment was 44.3 [33.8-55.4] mm. Median interslice distance was 0.40 mm. A total of 724 lesions were identified

Table 1. Baseline characteristics	
	n = 581 patients
Patient characteristics	
Age, years	61.6 ± 11.3
Men, n (%)	439 (75.6)
Diabetes Mellitus, n (%)	99 (17.0)
Hypertension. n (%)	300 (51.6)
Hypercholesterolemia, n (%)	321 (55.2)
Smoking, n (%)	169 (29.1)
Positive family history, n (%)	301 (51.8)
Previous MI, n (%)	184 (31.7)
Previous PCI, n (%)	186 (32.0)
Previous CABG, n (%)	18 (3.1)
Previous stroke, n (%)	26 (4.5)
History of peripheral artery disease, n (%)	36 (6.2)
History of renal insufficiency, n (%)	32 (5.5)
History of heart failure, n(%)	19 (3.3)
C-reactive protein, mg/L	2.1 [0.9-5.4]
Procedural characteristics	
Indication for angiography	
Acute MI, n (%)	167 (28.7)
Unstable angina, n (%)	151 (26.0)
Stable angina, n (%)	254 (43.7)
Other, n (%)	9 (1.5)
Coronary artery disease*	
No significant stenosis, n (%)	43 (7.4)
1-vessel disease, n (%)	308 (53.0)
2-vessel disease, n (%)	168 (28.9)
3-vessel disease, n (%)	62 (10.7)
PCI performed, n (%)	511 (88.0)
IVUS characteristics	
Imaged coronary artery	
Left anterior descending, n (%)	210 (36.1)
Left circumflex, n (%)	195 (33.6)
Right coronary artery, n (%)	176 (30.3)
Imaged segment length, mm	44.3 [33.8-55.4]

* A significant stenosis was defined as a stenosis ≥50% of vessel diameter by visual assessment on the coronary angiogram.
CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention.

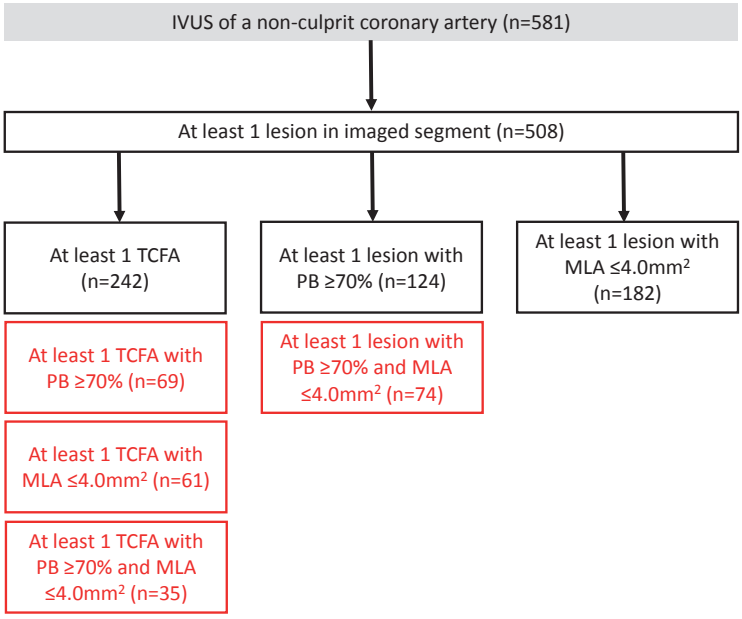


Figure 2. Study participants and presence of high risk coronary lesions on intravascular ultrasound
IVUS, intravascular ultrasound; MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma.

in the imaged coronary segment of 508 (87.4%) patients, including 127 (17.5%) lesions with a plaque burden of at least 70% in 124 (21.3%) patients and 206 (28.5%) lesions with a minimal luminal area of 4.0 mm^2 or less in 182 (31.3%) patients (Figure 2 and Supplemental table 1). On the basis of radiofrequency IVUS, 271 (37.4%) of the lesions have been classified as TCFA in 242 (41.7%) patients, including 71 (9.8%) TCFA lesions with a plaque burden of at least 70% in 69 (11.9%) patients, 61 (8.4%) TCFA lesions with a minimal luminal area of 4.0 mm^2 or less in 61 (10.5%) patients, and 35 (4.8%) TCFA lesions with a plaque burden of at least 70% and a minimal luminal area of 4.0 mm^2 in 35 (6.0%) patients. Antiplatelet medications and statins were prescribed to the majority of patients at time of discharge (Supplemental table 2).

Major adverse cardiac events

Vital status was complete for 580 (99.8%) patients. Response rate of the questionnaires that were sent to all living patients was 91.5%. After 1 year of follow-up, 56 patients had at least 1 event (Table 2). Unplanned coronary revascularization was performed in 4 patients who did not have PCI during the index procedure. A total of 11 patients had a definite culprit lesion related event, while 27 patients had a definite non-culprit lesion related event. Another 18 patients had an event that could not be judged to be

Table 2. Patients with major adverse cardiac events

	Definite culprit lesion related events	Definite non-culprit lesion related events	Indeterminate events	Non-culprit lesion related and indeterminate events combined	All events
Composite of MACE, n	11	27	18	45*	56
Death from any cause, n	1	1	16	17	18
Definite cardiac or unexplained death, n	1	1	6	7	8
Acute coronary syndrome, n	3	9	2	11	14
Myocardial infarction, n	2	3	2	5	7
Unplanned coronary revascularization, n	7	17	0	17	24
Composite of death or acute coronary syndrome, n	4	10	18	28**	32

* Primary endpoint
** Secondary endpoint

either culprit lesion related or non-culprit lesion related and were therefore classified as having an indeterminate event. The cumulative Kaplan-Meier incidence of the 30-day, 6-month and 1-year MACE (primary endpoint) was 0.7%, 4.7%, and 7.8%, respectively. The cumulative Kaplan-Meier incidence of the 30-day, 6-month and 1-year composite of death or ACS (secondary endpoint) was 0.7%, 3.1%, and 4.8%, respectively.

Associations with incident major adverse cardiac events

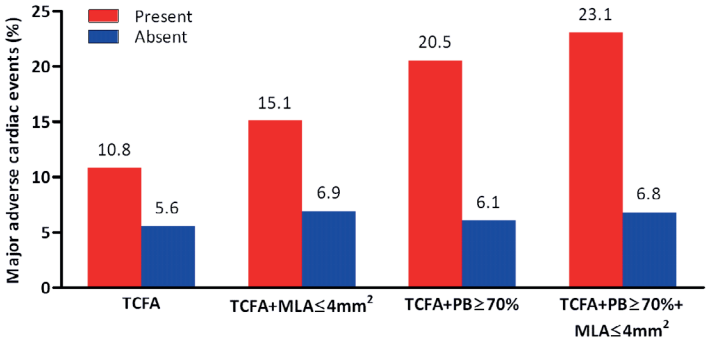
Patients who did not had any lesion in the imaged coronary segment seemed to have lower occurrence of MACE (absent 4.1% vs. present 8.3%; HR 0.48, 95% CI 0.15-1.54; p=0.22) and lower occurrence of the composite of death or ACS only (absent 1.4% vs. present 5.4%; HR 0.25, 95% CI 0.034-1.83; p=0.17), although these associations were not statistically significant. The amount of necrotic core in the imaged coronary segment was associated with MACE (Supplemental table 3).

After adjustment for clinical characteristics, the presence of TCFA lesions (present 10.8% vs. absent 5.6%; adjusted HR 1.98, 95% CI 1.09-3.60; p=0.026) and lesions with a plaque burden of at least 70% (present 16.2% vs. absent 5.5%; adjusted HR 2.90, 95% CI 1.60-5.25; p<0.001) were independently associated with higher occurrence of MACE, while the presence of lesions with a minimal luminal area of 4.0 mm^2 or less was not (present 9.4% vs. absent 7.1%; adjusted HR 1.23, 95% CI 0.67-2.26; p=0.50) (Table 3 and Supplemental table 4). There was no heterogeneity in the HR estimates between patients admitted with and without ACS (heterogeneity p=0.31 for TCFA, p=0.58 for plaque burden of at least 70% and p=0.65 for minimal luminal area of 4.0 mm^2 or less). Calcified TCFA lesions seemed to carry higher risk than non-calcified TCFA lesions, although the

Table 3. Associations with major adverse cardiac events

	Unadjusted model	P value	Age and gender adjusted model	P value	Age, gender and indication for angiography adjusted model	P value	Full model*	P value
Major adverse cardiac events (primary endpoint)								
Thin-cap fibroatheroma	HR 1.96 (1.08-3.53)	0.026	HR 1.97 (1.09-3.57)	0.024	HR 2.00 (1.10-3.62)	0.022	HR 1.98 (1.09-3.60)	0.026
Plaque burden ≥70%	HR 3.15 (1.75-5.68)	<0.001	HR 2.83 (1.57-5.13)	0.001	HR 2.83 (1.56-5.12)	0.001	HR 2.90 (1.60-5.25)	<0.001
MLA ≤4.0mm^2	HR 1.36 (0.74-2.48)	0.32	HR 1.24 (0.68-2.28)	0.48	HR 1.24 (0.68-2.28)	0.48	HR 1.23 (0.67-2.26)	0.50
Composite of death or acute coronary syndrome (secondary endpoint)								
Thin-cap fibroatheroma	HR 2.56 (1.18-5.54)	0.017	HR 2.60 (1.20-5.64)	0.015	HR 2.54 (1.17-5.51)	0.019	HR 2.51 (1.15-5.49)	0.021
Plaque burden ≥70%	HR 2.11 (0.97-4.56)	0.059	HR 1.90 (0.87-4.15)	0.11	HR 1.92 (0.88-4.20)	0.10	HR 2.01 (0.92-4.39)	0.079
MLA ≤4.0mm^2	HR 1.23 (0.57-2.67)	0.60	HR 1.12 (0.52-2.43)	0.78	HR 1.13 (0.52-2.45)	0.76	HR 1.14 (0.53-2.49)	0.73

* Variables entered into the full model were age, gender, diabetes mellitus, hypertension, history of percutaneous coronary intervention and indication for coronary angiography.
HR, hazard ratio, MLA, minimal luminal area



95% CI lower limit (%)	6.9	3.1	6.1	4.7	10.9	3.9	9.0	4.6
95% CI upper limit (%)	14.7	8.1	24.1	9.1	30.1	8.3	37.2	9.0
Prevalence (%)	41.7	58.3	10.5	89.5	11.9	88.1	6.0	94.0
No. at risk at 1 year (n)	211	312	50	473	52	471	52	471
Hazard ratio (95% CI)	1.96 (1.08-3.53)		2.26 (1.09-4.69)		3.47 (1.86-6.49)		3.70 (1.72-7.95)	
P value	0.024		0.025		<0.001		<0.001	

Figure 3. Cumulative Kaplan-Meier incidence estimates of major adverse cardiac events
Percentages are 1-year cumulative Kaplan-Meier incidence estimates. Hazard ratios are estimated using univariate Cox proportional hazards regression analysis. P values are obtained with log-rank test.
MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma.

difference was not statistically significant (p=0.32) (Supplemental figure 1). The presence of TCFA lesions was also significantly associated with the composite of death or ACS only (present 7.5% vs. absent 3.0%; adjusted HR 2.51, 95% CI 1.15-5.49; p=0.021).

Risk for occurrence of MACE was further increased if the TCFA lesions had a minimal luminal area of 4.0 mm^2 or less, had a plaque burden of at least 70%, or a combination of these three characteristics (Figure 3 and Supplemental figure 2). TCFA lesions with a plaque burden of at least 70% were associated with higher MACE rate both in the first 6 months (p=0.011) and after 6 months (p<0.001) of follow-up, while smaller TCFA lesions were only associated with higher MACE rate after 6 months (p=0.033) (Figure 4).

DISCUSSION

This study investigated the prognostic value of *in-vivo* high risk plaque detection by IVUS for the occurrence of MACE in patients undergoing coronary angiography. In line with previous studies, we found that the presence of a TCFA lesion as assessed by IVUS in a non-culprit coronary artery was independently predictive for occurrence of MACE that was not related to the index procedure.(10, 14) The event rate was even further increased when patients had a TCFA lesion with a minimal luminal area of 4.0 mm^2 or less, a plaque

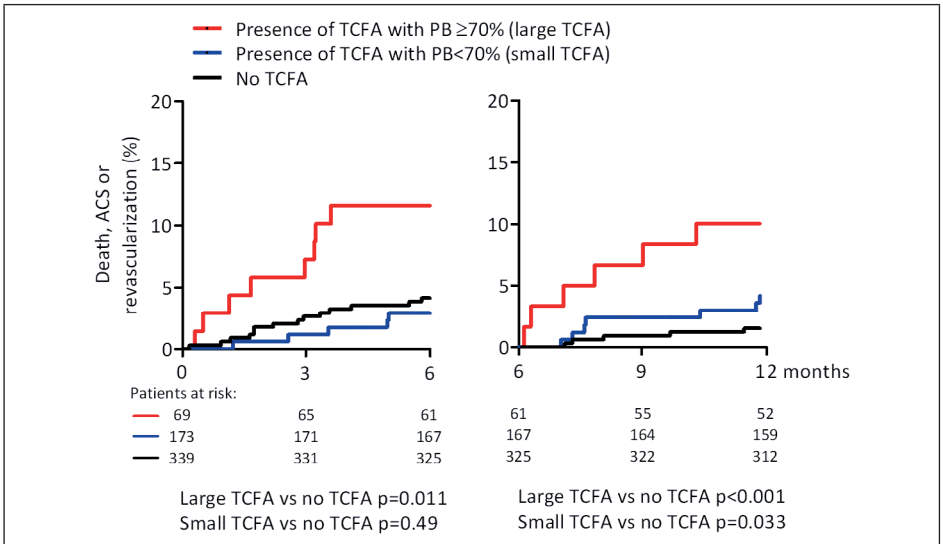


Figure 4. Associations with of short-term and long-term major adverse cardiac events
P values are obtained with log-rank test. Overall P value 0-6 months is 0.009; overall P value 6-12 months is 0.002.
PB, plaque burden; TCFA, thin-cap fibroatheroma.

burden of at least 70%, or a combination thereof. Our study is the *first* to demonstrate that the presence of such vulnerable coronary lesions as assessed *in-vivo* by IVUS are significantly associated with the occurrence of acute cardiac events (composite of death or ACS only) that were not related to the index procedure. Furthermore, we found that patients with a large TCFA lesion (with a plaque burden of at least 70%) were at higher risk than patients with a small TCFA lesion. The presence of a small TCFA lesion was only predictive for clinical events occurring on the longer term (after 6 months).

Although the PROSPECT and the Virtual histology Intravascular ultrasound in Vulnerable Atherosclerosis (VIVA) studies have previously reported on the prognostic value of vulnerable plaque detection by IVUS, there are some limitations to the conclusion of these studies.(10, 14) First, the PROSPECT study only enrolled ACS patients. Therefore, the conclusions of this study cannot be directly extrapolated to patients with stable angina. In contrast, our study presents a patient population that underwent coronary angiography for ACS or stable angina and that may better reflect the "real world" clinical practice. Second, the vast majority of events in the PROSPECT study consisted of rehospitalizations for unstable or progressive angina (69 out of the 74 patients with primary composite endpoint), while the majority of events in the VIVA study consisted of coronary revascularizations (14 out of the 16 patients with primary composite endpoint). Our study demonstrated that vulnerable coronary lesions as assessed *in-vivo* by IVUS are significantly associated with the occurrence of acute cardiac events (composite of death

or ACS only) that were not related to the index procedure. Finally, an important difference is that IVUS was performed in three coronary vessels in the PROSPECT and VIVA studies. Our study demonstrated that IVUS in only one non-culprit vessel is sufficient for prognostication. This finding is relevant for the use of IVUS in daily clinical practice, since IVUS acquisition and analysis of three vessels is more time consuming and may increase risk for complications.

Previous studies have demonstrated that coronary atherosclerotic plaque burden as assessed with coronary computed tomography angiography or IVUS is associated with progression of the lesion and with incident clinical events during follow-up.(15-17) Similarly, the PROSPECT and the VIVA studies have shown that lesions with a plaque burden of at least 70% were strongly associated with their primary endpoint.(10, 14) In the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology (PREDICTION) study, large plaque burden and low local endothelial shear stress were also independently associated with progression of the lesion and narrowing of the lumen.(18) In accordance with these observations, we found that patients with a coronary lesion that had a plaque burden of at least 70% were at higher risk for MACE. However, the presence of a lesion with a plaque burden of at least 70% was not significantly predictive for the composite of death or ACS only. These findings suggest that lesions with a high plaque burden are at high risk to cause a flow-limiting stenosis, requiring coronary revascularizations and rehospitalizations for progressive angina.

TCFA is the most common pathological substrate of ACS and has been found to be associated with incident cardiac events.(19) In the PROSPECT study, non-culprit lesions associated with recurrent events (mainly driven by rehospitalizations) were more likely to be classified as TCFA on the basis of radiofrequency IVUS (adjusted HR 3.35, 95% CI, 1.77-6.36; p<0.001).(10) In the VIVA study, presence of a non-calcified TCFA lesion was the only factor that was associated with MACE, which was mainly driven by coronary revascularizations (unadjusted HR 1.79; 95% CI 1.20-2.66, p=0.004).(14) Likewise, we found that the presence of TCFA lesions as assessed with IVUS was independently predictive for MACE (adjusted HR 1.98, 95% CI 1.09-3.60; p=0.026). Furthermore, the predictive value of TCFA lesions for occurrence of acute cardiac events (composite of death or ACS only) was even stronger (adjusted HR 2.51, 95% CI 1.15-5.49; p=0.021). These findings emphasize the biological importance of TCFA for plaque rupture.

We have also found that patients with a large TCFA lesion (with a plaque burden of at least 70%) were at higher risk than patients with a small TCFA lesion. Furthermore, large TCFA lesions were associated with higher MACE rate within and after 6 months of follow-up, while smaller TCFA lesions were only associated with higher MACE rate after 6 months. Based on these observations, it can be hypothesized that large TCFA lesions are more vulnerable and more prone to rupture, while small TCFA lesions may grow in time

and may become more vulnerable in the future. In line with our findings, two previous studies have demonstrated that the majority of the untreated non-culprit TCFA lesions retain their TCFA morphology during follow-up (6 to 13 months), and may be accompanied by a decrease in minimal luminal area and an increase in necrotic core.(20-21) An other small study of patients with a lower risk profile, however, has demonstrated that the majority of the TCFA lesions were healed after 1 year.(22)

Different MACE definitions have been used in the above mentioned studies (death, ACS and unplanned revascularization in our study; cardiovascular death, cardiac arrest, myocardial infarction and rehospitalization due to unstable or progressive angina in the PROSPECT study; death, myocardial infarction, and unplanned revascularization in the VIVA study).(10, 14) Therefore, MACE rates of these studies cannot be directly compared. Nevertheless, the incidence of MACE seemed to be relatively high in our study population. For example, 18 deaths occurred in 581 patients within 1 year in our study compared to 2 deaths in 170 patients within 625 days in the VIVA, 31 deaths in 697 patients within 3.4 years in the PROSPECT and 4 deaths in 506 patients within 9 months in the PREDICTION study.(10, 14, 18) However, the MACE rate in our study was consistent with that of previous "all-comer" registries in our hospital, which further emphasizes that our study population may better reflect the "real world" clinical practice.(23-24)

Some limitations of this study need to be acknowledged. Firstly, this is a prospective observational cohort study. Although we aimed to include a patient population that reflects clinical practice, those patients with any of the exclusion criteria could not be included in this study.(11) Secondly, the spatial resolution of IVUS virtual histology (150µm) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65µm).(25) Therefore IVUS virtual histology tends to over-estimate the number of TCFA lesions. Nevertheless, the presence of IVUS virtual histology detected TCFA lesions has prognostic information and is therefore clinically relevant. Thirdly, the relatively small number of endpoints did not allow us to evaluate whether adding IVUS imaging to a prognostic model with conventional risk factors would result in improved risk prediction. Finally, repeat intracoronary imaging with IVUS virtual histology was not performed. Therefore, the dynamic nature of coronary artery lesion morphology could not be investigated. Large, future studies (e.g. IBIS-3, www.trialregister.nl identifier NTR2872) may provide useful data in this respect.(26)

In conclusion, IVUS virtual histology appeared to be a useful tool for *in-vivo* detection of high risk coronary lesions. In patients undergoing coronary angiography, the presence of IVUS virtual histology-derived TCFA lesions in a non-culprit coronary artery is strongly and independently predictive for occurrence of MACE, particularly of death and ACS. TCFA lesions with a large plaque burden are of higher risk than small TCFA lesions, especially on the short-term.

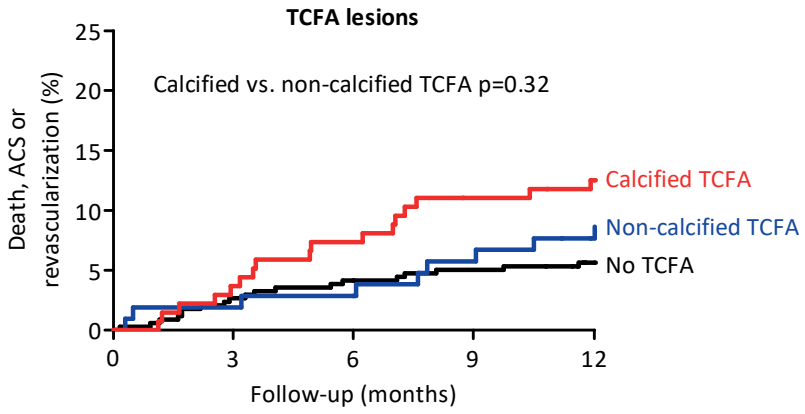
REFERENCES

1. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott M, Meigs J, Mozaffarian D, Nichol G, O'Donnell C, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Steinberger J, Thom T, Wasserthiel-Smoller S, Wong N, Wylie-Rosett J, Hong Y. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2009; 119:480-486.
2. Smith SC, Jr., Benjamin EJ, Bonow RO, Braun LT, Creager MA, Franklin BA, Gibbons RJ, Grundy SM, Hiratzka LF, Jones DW, Lloyd-Jones DM, Minissian M, Mosca L, Peterson ED, Sacco RL, Spertus J, Stein JH, Taubert KA. AHA/ACCF Secondary Prevention and Risk Reduction Therapy for Patients with Coronary and other Atherosclerotic Vascular Disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation. *Circulation* 2011; 124:2458-2473.
3. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol* 2006; 47:C13-18.
4. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Jr., Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation* 2003; 108:1664-1672.
5. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Jr., Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation* 2003; 108: 1772-1778.
6. Glaser R, Selzer F, Faxon DP, Laskey WK, Cohen HA, Slater J, Detre KM, Wilensky RL. Clinical progression of incidental, asymptomatic lesions discovered during culprit vessel coronary intervention. *Circulation* 2005; 111:143-149.
7. Nair A, Margolis MP, Kuban BD, Vince DG. Automated coronary plaque characterisation with intravascular ultrasound backscatter: ex vivo validation. *EuroIntervention* 2007; 3:113-120.
8. Garcia-Garcia HM, Mintz GS, Lerman A, Vince DG, Margolis MP, van Es GA, Morel MA, Nair A, Virmani R, Burke AP, Stone GW, Serruys PW. Tissue characterisation using intravascular radiofrequency data analysis: recommendations for acquisition, analysis, interpretation and reporting. *EuroIntervention* 2009; 5:177-189.
9. Rodriguez-Granillo GA, Garcia-Garcia HM, Mc Fadden EP, Valgimigli M, Aoki J, de Feyter P, Serruys PW. In vivo intravascular ultrasound-derived thin-cap fibroatheroma detection using ultrasound radiofrequency data analysis. *J Am Coll Cardiol* 2005; 46:2038-2042.

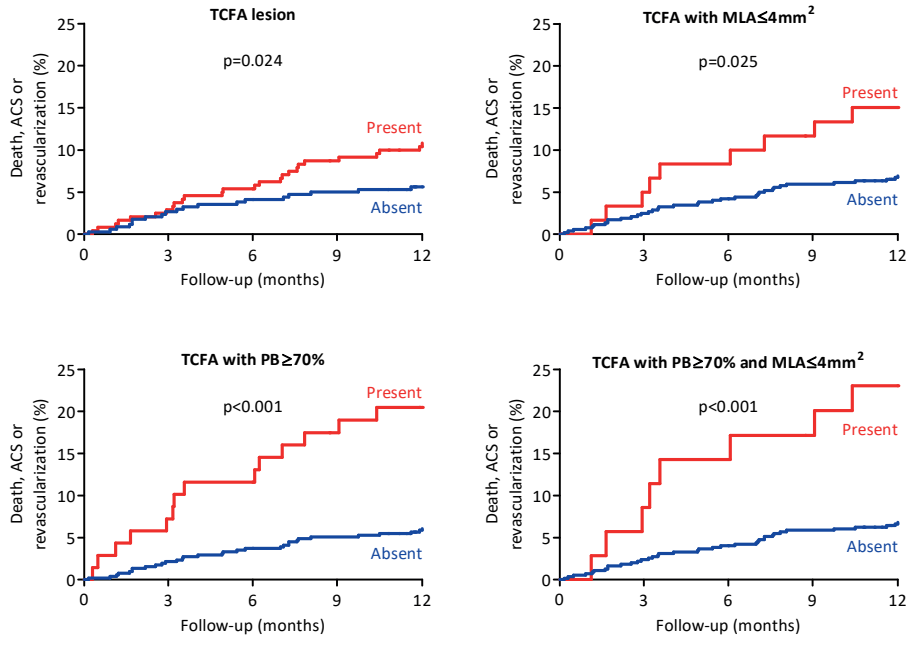
10. Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med* 2011; 364:226-235.
11. De Boer SPM, Cheng JM, Garcia-Garcia HM, Oemrawsingh RM, Van Geuns RJ, Regar E, Zijlstra F, Laaksonen R, Halperin E, Kleber ME, Koenig W, Boersma E, Serruys PW. Relation of genetic profile and novel circulating biomarkers with coronary plaque phenotype as determined by intravascular ultrasound: Rationale and design of the ATHEROREMO-IVUS study. *EuroIntervention* 2013; published online ahead of print doi:pii:20130113-01.
12. Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheye S, Aschermann M, Duckers H, Bleie O, Dudek D, Botker HE, von Birgelen C, D'Amico D, Hutchinson T, Zambanini A, Mastik F, van Es GA, van der Steen AF, Vince DG, Ganz P, Hamm CW, Wijns W, Zalewski A. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation* 2008; 118:1172-1182.
13. Erhardt L, Herlitz J, Bossaert L, Halinen M, Keltai M, Koster R, Marcassa C, Quinn T, van Weert H. Task force on the management of chest pain. *Eur Heart J* 2002; 23:1153-1176.
14. Calvert PA, Obaid DR, O'Sullivan M, Shapiro LM, McNab D, Densem CG, Schofield PM, Braganza D, Clarke SC, Ray KK, West NE, Bennett MR. Association between IVUS findings and adverse outcomes in patients with coronary artery disease: the VIVA (VH-IVUS in Vulnerable Atherosclerosis) Study. *JACC Cardiovasc imaging* 2011; 4:894-901.
15. Nicholls SJ, Hsu A, Wolski K, Hu B, Bayturan O, Lavoie A, Uno K, Tuzcu EM, Nissen SE. Intravascular ultrasound-derived measures of coronary atherosclerotic plaque burden and clinical outcome. *J Am Coll Cardiol* 2010; 55:2399-2407.
16. Min JK, Dunning A, Lin FY, Achenbach S, Al-Mallah M, Budoff MJ, Cademartiri F, Callister TQ, Chang HJ, Cheng V, Chinnaiyan K, Chow BJ, Delago A, Hadamitzky M, Hausleiter J, Kaufmann P, Maffei E, Raff G, Shaw LJ, Villines T, Berman DS. Age- and sex-related differences in all-cause mortality risk based on coronary computed tomography angiography findings results from the International Multicenter CONFIRM (Coronary CT Angiography Evaluation for Clinical Outcomes: An International Multicenter Registry) of 23,854 patients without known coronary artery disease. *J Am Coll Cardiol* 2011; 58:849-860.
17. Papadopoulou SL, Neefjes LA, Garcia-Garcia HM, Flu WJ, Rossi A, Dharampal AS, Kitslaar PH, Mollet NR, Veldhof S, Nieman K, Stone GW, Serruys PW, Krestin GP, de Feyter PJ. Natural history of coronary atherosclerosis by multislice computed tomography. *JACC Cardiovasc Imaging* 2012; 5: S28-37.
18. Stone PH, Saito S, Takahashi S, Makita Y, Nakamura S, Kawasaki T, Takahashi A, Katsuki T, Namiki A, Hirohata A, Matsumura T, Yamazaki S, Yokoi H, Tanaka S, Otsuji S, Yoshimachi F, Honye J, Harwood D, Reitman M, Coskun AU, Papafaklis MI, Feldman CL. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation* 2012; 126:172-181.
19. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000; 20:1262-1275.
20. Zhao Z, Witzenbichler B, Mintz GS, Jaster M, Choi SY, Wu X, He Y, Margolis MP, Dressler O, Cristea E, Parise H, Mehran R, Stone GW, Maehara A. Dynamic nature of nonculprit coronary artery lesion morphology in STEMI: a serial IVUS analysis from the HORIZONS-AMI trial. *JACC Cardiovasc Imaging* 2013; 6:86-95.

21. Diletti R, Garcia-Garcia HM, Gomez-Lara J, Brugaletta S, Wykrzykowska JJ, van Ditzhuijzen N, van Geuns RJ, Regar E, Ambrosio G, Serruys PW. Assessment of coronary atherosclerosis progression and regression at bifurcations using combined IVUS and OCT. *JACC Cardiovasc Imaging* 2011; 4: 774-780.
22. Kubo T, Maehara A, Mintz GS, Doi H, Tsujita K, Choi SY, Katoh O, Nasu K, Koenig A, Pieper M, Rogers JH, Wijns W, Bose D, Margolis MP, Moses JW, Stone GW, Leon MB. The dynamic nature of coronary artery lesion morphology assessed by serial virtual histology intravascular ultrasound tissue characterization. *J Am Coll Cardiol* 2010; 55:1590-1597.
23. Lemos PA, Serruys PW, van Domburg RT, Saia F, Arampatzis CA, Hoye A, Degertekin M, Tanabe K, Daemen J, Liu TK, McFadden E, Sianos G, Hofma SH, Smits PC, van der Giessen WJ, de Feyter PJ. Unrestricted utilization of sirolimus-eluting stents compared with conventional bare stent implantation in the "real world": the Rapamycin-Eluting Stent Evaluated At Rotterdam Cardiology Hospital (RESEARCH) registry. *Circulation* 2004; 109:190-195.
24. Ong AT, Serruys PW, Aoki J, Hoye A, van Mieghem CA, Rodriguez-Granillo GA, Valgimigli M, Sonnenschein K, Regar E, van der Ent M, de Jaegere PP, McFadden EP, Sianos G, van der Giessen WJ, de Feyter PJ, van Domburg RT. The unrestricted use of paclitaxel- versus sirolimus-eluting stents for coronary artery disease in an unselected population: one-year results of the Taxus-Stent Evaluated at Rotterdam Cardiology Hospital (T-SEARCH) registry. *J Am Coll Cardiol* 2005; 45:1135-1141.
25. Garcia-Garcia HM, Costa MA, Serruys PW. Imaging of coronary atherosclerosis: intravascular ultrasound. *Eur Heart J* 2010; 31:2456-2469.
26. Simsek C, Garcia-Garcia HM, van Geuns RJ, Magro M, Girasis C, van Mieghem N, Lenzen M, de Boer S, Regar E, van der Giessen W, Raichlen J, Duckers HJ, Zijlstra F, van der Steen T, Boersma E, Serruys PW. The ability of high dose rosuvastatin to improve plaque composition in non-intervened coronary arteries: rationale and design of the Integrated Biomarker and Imaging Study-3 (IBIS-3). *EuroIntervention* 2012; 8:235-241.

SUPPLEMENTAL FIGURES AND TABLES



Supplemental figure 1. Cumulative Kaplan-Meier event curves stratified by presence of thin-cap fibroatheroma lesions
P value is obtained with log-rank test.
ACS indicates acute coronary syndrome; TCFA, thin-cap fibroatheroma.



Supplemental figure 2. Cumulative Kaplan-Meier event curves stratified by presence of thin-cap fibroatheroma lesions in combination with other high-risk lesions types.
P values are obtained with log-rank test.
ACS indicates acute coronary syndrome; MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma.

Supplemental table 1. Lesion types classified with intravascular ultrasound virtual histology	
	n = 724 lesions
1. Adaptive intimal thickening, n (%)	0 (0.0)
2. Pathological intimal thickening, n (%)	39 (5.4)
3. Fibrotic plaque, n (%)	122 (16.9)
4. Fibrocalcific plaque, n (%)	112 (15.5)
5. Fibroatheroma, n (%)	58 (8.0)
6. Calcified fibroatheroma, n (%)	122 (16.9)
7. Thin-cap fibroatheroma, n (%)	128 (17.7)
8. Calcified thin-cap fibroatheroma, n (%)	143 (19.8)

Supplemental table 2. Medication use at discharge	
	n = 581 patients
Aspirin, n (%)	556 (95.7)
Thienopyridine, n (%)	543 (93.5)
Statin, n (%)	515 (88.6)
Beta blocker, n (%)	441 (75.9)
ACE inhibitor or ARB, n (%)	388 (66.8)

Presented medication use was at time of discharge from our hospital. Patients may be discharged to a regional hospital for further treatment.
ACE indicates angiotensin converting enzyme; ARB, angiotensin receptor blocker.

Supplemental table 3. Association between necrotic core in imaged coronary segment and non-culprit lesion related and indeterminate major adverse cardiac events		
	Unadjusted HR (95%CI)	**P value**
Necrotic core percentage	1.14 (0.80-1.64)*	0.48
Necrotic core volume	1.65 (1.09-2.51)**	0.018

* Unadjusted hazard ratio per 10% increase in necrotic core.
** Unadjusted hazard ratio per standard deviation increase in ln-transformed necrotic core volume.

Supplemental table 4. Associations with non-culprit lesion related and indeterminate major adverse cardiac events

	Unadjusted model		Multivariable model 1		Multivariable model 2		Full model	
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Major adverse cardiac events (primary endpoint)								
TCFA	1.96 (1.08-3.53)	0.026	1.97 (1.09-3.57)	0.024	2.00 (1.10-3.62)	0.022	1.98 (1.09-3.60)	0.026
Age			1.04 (1.02-1.07)	0.002	1.04 (1.01-1.07)	0.003	1.04 (1.01-1.07)	0.003
Sex			1.15 (0.58-2.27)	0.70	1.13 (0.57-2.24)	0.73	1.10 (0.55-2.21)	0.78
Indication					1.16 (0.64-2.09)	0.63	0.98 (0.52-1.84)	0.95
Diabetes							1.63 (0.83-3.19)	0.16
Hypertension							0.93 (0.50-1.72)	0.81
Prior PCI							1.54 (0.82-2.89)	0.18
PB ≥70%	3.15 (1.75-5.68)	<0.001	2.83 (1.57-5.13)	0.001	2.83 (1.56-5.12)	0.001	2.90 (1.60-5.25)	<0.001
Age			1.04 (1.01-1.07)	0.008	1.04 (1.01-1.07)	0.008	1.04 (1.01-1.07)	0.009
Sex			1.10 (0.55-2.19)	0.79	1.09 (0.55-2.18)	0.80	1.07 (0.54-2.14)	0.85
Indication					1.05 (0.58-1.90)	0.86	0.84 (0.44-1.60)	0.60
Diabetes							1.63 (0.83-3.18)	0.16
Hypertension							0.99 (0.54-1.82)	0.98
Prior PCI							1.67 (0.88-3.16)	0.12
MLA ≤4.0mm^2	1.36 (0.74-2.48)	0.32	1.24 (0.68-2.28)	0.48	1.24 (0.68-2.28)	0.48	1.23 (0.67-2.26)	0.50
Age			1.04 (1.01-1.07)	0.003	1.04 (1.01-1.07)	0.003	1.04 (1.01-1.07)	0.004
Sex			1.18 (0.59-2.34)	0.64	1.17 (0.59-2.33)	0.66	1.14 (0.57-2.30)	0.71
Indication					1.07 (0.59-1.94)	0.82	0.89 (0.47-1.67)	0.71
Diabetes							1.64 (0.84-3.20)	0.15
Hypertension							1.00 (0.54-1.84)	0.99
Prior PCI							1.57 (0.83-2.96)	0.17

Supplemental table 4 (continued)

	Unadjusted model		Multivariable model 1		Multivariable model 2		Full model	
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Composite of death or acute coronary syndrome (secondary endpoint)								
TCFA	2.56 (1.18-5.54)	0.017	2.60 (1.20-5.64)	0.015	2.54 (1.17-5.51)	0.019	2.51 (1.15-5.49)	0.021
Age			1.05 (1.01-1.08)	0.008	1.05 (1.01-1.08)	0.007	1.05 (1.02-1.09)	0.005
Sex			0.77 (0.35-1.72)	0.53	0.80 (0.36-1.79)	0.58	0.75 (0.33-1.69)	0.49
Indication					0.74 (0.34-1.59)	0.44	0.58 (0.26-1.32)	0.19
Diabetes							1.34 (0.53-3.37)	0.54
Hypertension							0.75 (0.34-1.64)	0.47
Prior PCI							2.29 (1.04-5.05)	0.04
PB ≥70%	2.11 (0.97-4.56)	0.059	1.90 (0.87-4.15)	0.11	1.92 (0.88-4.20)	0.10	2.01 (0.92-4.39)	0.079
Age			1.04 (1.01-1.08)	0.015	1.05 (1.01-1.08)	0.011	1.05 (1.01-1.09)	0.007
Sex			0.77 (0.34-1.72)	0.52	0.81 (0.36-1.82)	0.61	0.75 (0.33-1.69)	0.48
Indication					0.67 (0.31-1.44)	0.30	0.48 (0.21-1.10)	0.084
Diabetes							1.32 (0.53-3.30)	0.56
Hypertension							0.80 (0.37-1.72)	0.57
Prior PCI							2.55 (1.14-5.74)	0.023
MLA ≤4.0mm^2	1.23 (0.57-2.67)	0.60	1.12 (0.52-2.43)	0.78	1.13 (0.52-2.45)	0.76	1.14 (0.53-2.49)	0.73
Age			1.05 (1.01-1.08)	0.010	1.05 (1.01-1.09)	0.007	1.05 (1.02-1.09)	0.005
Sex			0.81 (0.36-1.80)	0.60	0.85 (0.38-1.91)	0.70	0.78 (0.34-1.77)	0.55
Indication					0.67 (0.31-1.45)	0.31	0.50 (0.22-1.14)	0.098
Diabetes							1.31 (0.52-3.28)	0.57
Hypertension							0.81 (0.37-1.75)	0.59
Prior PCI							2.46 (1.10-5.51)	0.029

HR indicates hazard ratio; MLA, minimal luminal area; PB, plaque burden, PCI, percutaneous coronary intervention; TCFA, thin-cap fibroatheroma.