CORONARY VULNERABILITY

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Coronary Vulnerability

Coronaire Vulnerabiliteit

Proefschrift

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Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels
en volgens besluit van het College voor Promoties.

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Coronary Vulnerability

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Thesis

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Erasmus University Rotterdam
by command of the
rector magnificus

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या देवी सर्वभूतेषु बुद्धिरूपेण संस्थिता ।
या देवी सर्वभूतेषु शक्तिरूपेण संस्थिता ।
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या देवी सर्वभूतेषु मातृरूपेण संस्थिता ।
नमस्तस्यै नमस्तस्यै नमस्तस्यै नमो नमः

To that Goddess who abides in all beings as intelligence
To that Goddess who abides in all beings as power
To that Goddess who abides in all beings as reflection
To that Goddess who abides in all beings as modesty
To that Goddess who abides in all beings as compassion
To that Goddess who abides in all beings as Mother
Salutations to Thee, again, again and again

Voor Hriday

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PROLOGUE

This manuscript describes an attempt of elucidating only a minute aspect of a complex problem, known as coronary artery disease (CAD). With an estimated 8 million deaths per year, CAD remains among the leading causes of premature death in the world, despite the fact that prevention, lifestyle interventions, pharmacologic strategies and revascularization have led to a decline in mortality rates over the past decades. Nevertheless, the fact that the number of life years lost to premature deaths is increasing in low- and middle-income regions is alarming.[1]

Patients with a formal diagnosis of CAD are at the scope of this thesis. For these patients, epidemiologists have been able to successfully create prediction models that aim to estimate the risk of death or myocardial infarction within a set timeframe. These models depend on the presence and recognition of traditional risk factors (such as hypertension, diabetes, smoking etc.) and cardiovascular history complemented by biometric factors. However, traditional cardiovascular risk factors are absent in a significant part of the population that nevertheless will develop CAD and its sequelae. In contrast, the prevalence of traditional risk factors is also high among the fraction of the population that will never endure a major adverse cardiovascular event (MACE) [2].

According to the key philosophy behind existing risk prediction models, the individual patient is considered to be a member of a group that is exposed to a certain (low-intermediate-high) constant risk over time, whereas the incidence of acute cardiovascular events is considered a random process, with event probabilities directly related to that group risk. Consequently, cardiovascular risk models usually predict reasonably well on a group level, but only poorly outline the course of individuals. [2] In addition, current risk prediction models do not account for the dynamic nature of the coronary pathophysiology. Individual patients with CAD actually do not have constant risks over time. Long periods of stability, with minimal plaque progression and low risk of cardiovascular events, are alternated by periods of increased plaque instability and rapid plaque progression, during which the risk of sudden plaque disruption and thrombotic coronary occlusion increases. [2]

Against this background, the common thread throughout parts 1 to 3 of this thesis is the search for improvement of risk prediction in patients with known CAD, i.e. more precise identification of those vulnerable for suffering a coronary event in the future.

Part 1, *"**Vulnerable Blood**"*, focusses on the additional value of several serum biomarkers for the prediction of MACE on a relatively long term (4 to 10 years of follow-up). These markers are traditionally measured once at the start of follow-up and hence assumed to reflect a constant cardiovascular risk, in a similar way as traditional risk models incorporate clinical risk factors.

Part 2, *"**Vulnerable Period**"*, focusses on serum biomarkers as well, but here the train of thought is more in line with the dynamics of coronary pathophysiology, i.e. that the risk of MACE within an individual patient is not constant, but variable over time. Hence, repeated biomarker measurements are explored in the BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS), in order to evaluate whether fluctuations in biomarker levels can predict the risk of an imminent MACE within the days to weeks to come.

In ***Part 3***, *"**Vulnerable Plaque**"*, the centre of interest is around invasive coronary imaging, including coronary angiography, intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS), for the prediction of MACE, as well as cross-sectional analyses evaluating the relation between these imaging techniques and serum biomarkers.

Accurate risk prediction is important to understand future risks of CAD patients, but clearly prediction alone will not alter their outcome. For that purpose, intervention studies are required in those deemed at high risk. Such studies, often combined with the search for those patient subsets to derive most benefit from the interventions, are described in ***Part 4***, *"**Intervention Studies**"*.

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CORONARY VULNERABILITY

VULNERABLE BLOOD

CORONARY VULNERABILITY

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2

MULTIMARKER RISK MODEL CONTAINING TROPONIN-T, INTERLEUKIN 10, MYELOPEROXIDASE AND PLACENTAL GROWTH FACTOR PREDICTS LONG-TERM CARDIOVASCULAR RISK AFTER NON-ST-SEGMENT ELEVATION ACUTE CORONARY SYNDROME

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ABSTRACT

Objective: To evaluate the predictive value of seven biomarkers, which individually have been shown to be independent predictors, for use in a combined multimarker model for long-term cardiovascular outcome after non-ST-segment elevation acute coronary syndrome (NSTEACS).

Design and setting: Levels of high-sensitivity C-reactive protein (hsCRP), myeloperoxidase, pregnancy-associated plasma protein A, placental growth factor (PlGF), soluble CD40 ligand (sCD40L), interleukin 10 (IL-10) and troponin-T (TnT) were determined in patients enrolled in the CAPTURE trial. Cox proportional hazard regression analyses were applied to evaluate the relation between biomarkers and the occurrence of all-cause mortality or non-fatal myocardial infarction (MI).

Patients: 1090 patients with NSTEACS.

Main outcome measure: All-cause mortality and non-fatal MI during a median follow-up of 4 years.

Results: The composite endpoint was reached by 15.3% of patients. Admission levels of TnT >0.01 µg/l (adjusted HR 1.8), IL-10 <3.5 ng/l (1.7), myeloperoxidase >350 µg/l (1.5) and PlGF >27 ng/l (1.9) remained significant predictors for the incidence of all-cause mortality or non-fatal MI after multivariable adjustment for other biomarkers and clinical characteristics, whereas hsCRP, pregnancy-associated plasma protein A and sCD40L were only associated with the endpoint in univariate analysis. A multimarker model consisting of TnT, IL-10, myeloperoxidase and PlGF predicted 4-year event rates that varied between 6.0% (all markers normal) and 35.8% (three or more biomarkers abnormal).

Conclusion: In patients with NSTEACS, biomarkers characterising distinct aspects of the underlying atherosclerotic process and myocardial damage of the initial cardiac event can assist in predicting long-term adverse cardiac outcomes. The use of combinations of selected biomarkers adds incremental predictive value to further risk stratification in an otherwise seemingly homogeneous NSTEACS population.

Atherosclerosis and plaque destabilisation leading to coronary thrombosis and an acute coronary syndrome (ACS) are the result of a very heterogeneous process, involving vascular inflammation, endothelial dysfunction and hypercoagulability.[1] Several novel serum biomarkers are thought to reflect these pathophysiological constituents of coronary artery disease (CAD) and have also proved to be independent predictors of future coronary events. C-reactive protein is the most extensively studied biomarker in this respect. It has been shown to be useful not only as a prognostic tool in patients with ACS,[2] but also in predicting the future risk of CAD in apparently healthy men and women.[3] Myeloperoxidase, a leucocytic enzyme that appears as part of the host defence in inflammatory disorders, and also present in soft plaque, was associated with an increased risk of major adverse cardiac events in patients with documented ACS,[2] as well as in those presenting with chest pain without evidence of myocardial necrosis.[4] Also expressed in ruptured and eroded plaques, but not in stable plaques, is the metalloproteinase pregnancy-associated plasma protein A (PAPP-A).[5,6] Placental growth factor (PlGF), a member of the vascular endothelial growth factor family, is considered a primary inflammatory instigator in atherosclerotic lesions,[7] and has prognostic value in patients with ACS.[8] In contrast, elevated levels of the anti-inflammatory cytokine interleukin 10 (IL-10) were associated with a lower risk of coronary events in patients with ACS and elevated high-sensitivity C-reactive protein (hsCRP) levels,[9] emphasising the importance of an inflammatory balance in the vascular wall. Finally, elevated levels of soluble CD40 ligand (sCD40L), which is primarily released from activated platelets,[10] were associated with an increased cardiovascular risk during 6 months of follow-up of ACS-patients.[11]

The prognostic value of these and other biomarkers for the risk of future cardiovascular events in ACS patients has previously been studied. These analyses typically assess each marker individually with adjustment for clinical patient characteristics.

In certain cases, two markers are combined in one model.[12] There are only two reports, however, in which the specific combination of three biomarkers provided incremental value for risk prediction after ACS.[13,14] Furthermore, the follow-up duration of previous multimarker studies in ACS patients was often limited to periods consisting of several months up to a maximum of 1 year after admission.[2,5,8,9,11,13-15] Nevertheless, coronary pathophysiology is sustained after acute interventional or pharmacological treatment and continuously triggers cardiovascular events during long-term follow-up. We therefore studied the relation between baseline levels of seven biomarkers, including troponin-T (TnT) as a marker of myocardial necrosis, both independently and in a combined multimarker risk model, and the incidence of allcause mortality or non-fatal myocardial infarction (MI) during an extended 4-year follow-up period in ACS patients who were enrolled in the CAPTURE trial.

METHODS

Patients and treatment

Patients admitted with unstable angina pectoris or non ST-elevation MI were eligible for CAPTURE if they had refractory unstable angina defined as: chest pain at rest with concomitant ECG abnormalities compatible with myocardial ischaemia (ST-segment-depression, ST-segment elevation, or abnormal T waves) and one or more episodes of typical chest pain, ECG abnormalities, or both, compatible with myocardial ischaemia during therapy with intravenous heparin and nitrates, started at least 2 h previously. The latest episode of ischaemia should have occurred within the 48 h before enrolment, corresponding to Braunwald class III unstable angina. All patients had undergone angiography and had significant CAD, with a culprit lesion suitable for percutaneous coronary intervention (PCI). Patients were enrolled within 24 h of coronary angiography and were randomly assigned to abciximab (ReoPro, Centocor BV, Leiden, The Netherlands; 0.25 mg/kg bolus plus 10 μg/min continuous infusion) or placebo after providing written informed consent. PCI was scheduled 18-24 h after the start of study medication. Study medication was started within 2 h of randomisation and continued until 1 h after the procedure. All patients received aspirin, heparin and nitrates, whereas β-blockers, calcium channel antagonists and other cardiovascular drugs were given at the discretion of the investigator.

Analytical techniques

Blood samples were drawn 8.7±4.9 h after the last episode of angina, but before PCI and before the incidence of adverse events. Serum and heparin plasma samples were available for the measurement of TnT, hsCRP, sCD40L, IL-10, myeloperoxidase, PAPP-A and PlGF levels. Biomarker measurements were performed blinded to the patients' histories. Levels of sCD40L, high-sensitivity IL-10, myeloperoxidase and PlGF were measured by ELISA (sCD40L, IL-10 and PlGF from R&D Systems, Wiesbaden, Germany and myeloperoxidase from Calbiochem, Merck KGaA, Darmstadt, Germany). Diagnostic thresholds were 5.0 μg/ l for sCD40L, 3.5 ng/l for IL-10, 350 μg/l for myeloperoxidase and 27 ng/l for PlGF. Levels of TnT and PAPP-A were determined using a electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Mannheim, Germany). A diagnostic threshold value of 0.01 μg/l for TnT and 12.6 mIU/l for PAPP-A was used. Levels of hsCRP were measured using the Behring BN II Nephelometer (Dade Behring, Deerfield, Illinois, USA). A diagnostic threshold value of 10 mg/l was used. All cut-off values were consistent with previous biomarker publications within this cohort in which the markers were described independently.[2,5,8,9,11,15]

Study endpoints

The endpoint of the present analysis was a composite of all-cause mortality and non-fatal MI during 4-year (median) follow-up. Follow-up at 6 months was part of the initial study protocol, and a clinical endpoint committee adjudicated these events.

MI during the index hospital stay was defined as values of creatine kinase or its myocardial type (MB) isoenzyme more than three times the upper limit of normal in at least two samples, with an increase by 50% over the previous value, or an ECG with new significant Q waves in two or more contiguous leads. MI after discharge was defined as concentrations of creatine kinase or its myocardial type isoenzyme above two times the upper limit of normal, or new significant Q waves in two or more contiguous ECG leads. Survival status and information on MI during extended follow-up (ie, after 6 months after randomisation) were obtained from the treating physician, the general practitioner, through self-reporting or municipal registries. These events were not adjudicated.

Data analysis

Continuous variables were summarised by median values with corresponding 25th and 75th percentiles. Discrete variables were summarised in terms of frequencies and percentages. Kaplan-Meier analyses were performed to evaluate the incidence of events over time. Univariable and multivariable Cox proportional hazards regression analyses were applied to evaluate the relation between all biomarkers and long-term outcome. In the multivariate model we adjusted variables known to be important predictors of outcome including age, gender, smoking, diabetes mellitus, hypertension, hypercholesterolaemia, left ventricular ejection fraction, ST-depression, ST-elevation or T-wave changes on the admittance ECG and history of MI, peripheral vascular disease, chronic heart failure or previous PCI. Crude and adjusted HR are presented with 95% CI. p Values were two-sided, with $p \leq 0.05$ being considered significant.

In case patients had more than one event (MI or death), the first was counted. In previous analyses of the CAPTURE study, significant interactions were observed between certain biomarkers (TnT and sCD40L) and allocated treatment with respect to the incidence of cardiovascular events during 6-month follow-up.[11,15] Formal statistical tests demonstrated that these interactions were no longer present with respect to the incidence of such events during long-term follow-up. Therefore, we decided to conduct all analyses on the patients allocated to placebo (N=544), as well as on the entire study population (placebo and abciximab combined; N=1090). The results of both sets of analyses are presented.

RESULTS

One thousand two hundred and sixty-five patients were enrolled in the CAPTURE trial. Multiple biomarker analysis proved feasible in 1090 patients. Baseline characteristics and clinical variables of these 1090 patients (546 abciximab, 544 placebo) who were included in our analyses are provided in table 1. The median follow-up duration was 47 months (25th and 75th percentile: 38, 55). The composite endpoint was reached in 167 (15.3%) patients (58 deaths and 109 non-fatal MIs).

Patients with elevated levels of most of the studied biomarkers had a higher risk of death or non-fatal MI than those with levels below the threshold (figure 1 and table 2), whereas elevated levels of IL-10 were associated with a better prognosis. For example, in the entire study population, those with TnT levels greater than 0.01 µg/l had a 20.3% incidence of death or nonfatal MI at 4 years of follow-up versus 11.1% in those with low TnT levels (unadjusted HR 2.1 and 95% CI 1.4 to 3.0). In patients receiving placebo, 4-year event rates were 23.5% and 11.6% in those with and without elevated TnT, respectively (unadjusted HR 2.2 and 95% CI 1.3 to 3.6; table 2). The results for all (other) biomarkers are given in table 2.

TnT, IL-10, myeloperoxidase and PlGF remained significant predictors for the incidence of all-cause mortality or non-fatal MI in the entire study population as well as in patients receiving placebo (table 2) after multivariable adjustment for clinical characteristics and all other biomarkers, whereas hsCRP, sCD40L and PAPP-A did not. Only two of the

Table 1. Baseline characteristics	
Age, years	62 (54, 69)
Male gender	73 (796)
Body mass index	26 (24, 28)
Diabetes mellitus	14 (153)
Hypercholesterolaemia	41 (447)
Current smoker or quitted within 1 year before	40 (436)
Previous angina	50 (545)
Previous MI	39 (425)
Previous heart failure	2 (22)
Peripheral artery disease	8 (87)
Previous coronary artery bypass graft	3 (33)
Previous PCI	13 (142)
History of any vascular disease	67 (730)
ST depression at presentation	43 (469)
Non-ST-elevation myocardial infarction at presentation	58 (632)

Age and body mass index are presented as median (IQR). All other data are presented in percentages (numbers). MI, myocardial infarction; PCI, percutaneous coronary intervention.

baseline clinical variables remained significant: age and ejection fraction (HR 1.03; 95% CI 1.01 to 1.04 per year and HR 0.98; 95% CI 0.96 to 0.99 per percentage point increase in ejection fraction, respectively; not given in table 2) in a multivariate model that included all biomarkers and baseline clinical variables.

We created a simple risk model for 4-year mortality and nonfatal MI by counting the presence or absence of an abnormal biomarker value that significantly predicted risk for an event. The percentages of patients with none, one, two or three or more abnormal biomarker levels were 5.2%, 22.1%, 43.5% and 29.2%, respectively. Four-year event rates varied between 6.0% (all markers normal) and 35.8% (three or more biomarkers

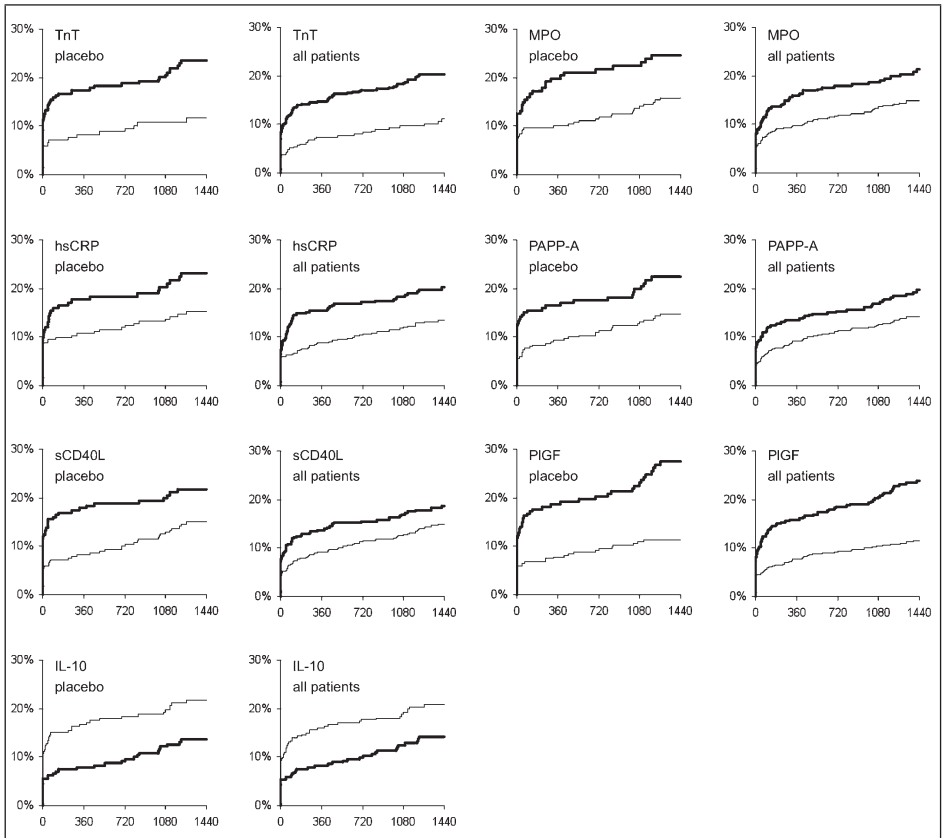

Figure 1. Kaplan-Meier curves for each independent biomarker.
Kaplan-Meier curves of the composite endpoint of all-cause mortality or non-fatal myocardial infarction during 4-year follow-up according to levels of different markers for placebo patients (left panels) and the entire study population (right panels). TnT, troponin-T (bold line indicates TnT >0.01 µg/l); hsCRP, high-sensitivity CRP (bold line is >10.0 mg/l); IL-10, interleukin 10 (bold line is ≥3.5 ng/l); MPO, myeloperoxidase (bold line is >350 µg/l); PAPP-A, pregnancy associated plasma protein A (bold line is >12.6 mIU/l); PlGF, placental growth factor (bold line is >27 ng/l); sCD40L, soluble CD40 ligand (bold line is >5.0 µg/l). The x-axis are days of follow-up.

Table 2. Relation between biomarkers of vascular inflammation, myocardial necrosis, platelet activation and a composite of all-cause mortality and non-fatal MI during 4-year follow-up

Biomarker	Result	Events at 4-year follow-up, % (Kaplan-Meier estimate)*		HR and 95% CI					
				Patients receiving placebo (N=544)			All patients (N=1090)		
		Placebo	All patients	Unadjusted	Adjusted, for all other biomarkers only†	Adjusted, for all other biomarkers and clinical characteristics¶	Unadjusted	Adjusted, for all other biomarkers only†	Adjusted, for all other biomarkers and clinical characteristics¶
TnT	>0.01 µg/l	23.5	20.3	2.2 (1.3 to 3.6)	2.3 (1.4 to 3.9)	2.0 (1.1 to 3.6)	2.1 (1.4 to 3.0)	2.1 (1.4 to 3.0)	1.8 (1.2 to 2.6)
	≤0.01 µg/l	11.6	11.1	1	1	1	1	1	1
hsCRP	>10 mg/l	23.0	20.3	1.6 (1.0 to 2.4)	*0.9 (0.5 to 1.4)*	*0.8 (0.5 to 1.3)*	1.6 (1.2 to 2.3)	*1.1 (0.8 to 1.6)*	*1.0 (0.7 to 1.5)*
	≤10 mg/l	15.3	13.5	1	1	1	1	1	1
sCD40L	>5.0 µg/l	21.8	18.7	1.6 (1.1 to 2.4)	*1.5 (0.9 to 2.3)*	*1.5 (0.9 to 2.3)*	1.4 (1.0 to 1.8)	*1.3 (0.9 to 1.7)*	*1.2 (0.9 to 1.6)*
	≤5.0 µg/l	15.1	15.0	1	1	1	1	1	1
IL-10	<3.5 ng/l	21.7	20.8	1.7 (1.1 to 2.6)	1.6 (1.1 to 2.5)	1.7 (1.1 to 2.7)	1.7 (1.1 to 2.5)	1.6 (1.1 to 2.5)	1.7 (1.1 to 2.6)
	≥3.5 ng/l	13.7	14.2	1	1	1	1	1	1
Myeloperoxidase	>350 µg/l	24.6	21.4	1.7 (1.1 to 2.5)	1.8 (1.2 to 2.9)	1.6 (1.0 to 2.6)	1.5 (1.1 to 2.1)	1.5 (1.1 to 2.1)	1.5 (1.1 to 2.1)
	≤350 µg/l	15.9	14.9	1	1	1	1	1	1
PAPP-A	>12.6 mIU/l	22.4	19.7	1.6 (1.1 to 2.5)	*1.3 (0.8 to 1.9)*	*1.2 (0.8 to 1.9)*	1.4 (1.1 to 1.9)	*1.1 (0.8 to 1.6)*	*1.1 (0.8 to 1.6)*
	≤12.6 mIU/l	14.7	14.1	1	1	1	1	1	1
PlGF	>27 ng/l	27.6	24.0	2.6 (1.7 to 3.9)	2.4 (1.6 to 3.7)	2.4 (1.4 to 4.1)	2.2 (1.6 to 3.0)	2.0 (1.4 to 2.8)	1.9 (1.3 to 2.8)
	≤27 ng/l	11.3	11.5	1	1	1	1	1	1

Non-significant results are reported in Italics.

*Events include all-cause mortality and non-fatal myocardial infarction (MI).

†All biomarkers as presented in this table.

¶All biomarkers as presented in this table, as well as index diagnosis, age, gender, smoking, diabetes mellitus, hypertension, left ventricular ejection fraction and hypercholesterolaemia, ST-depression, ST-elevation or T-wave changes on the admittance ECG and history of myocardial infarction, peripheral vascular disease, chronic heart failure or previous percutaneous coronary intervention.

TnT, troponin-T; hsCRP, high-sensitivity C-reactive protein; IL-10, interleukin-10; PAPP-A, pregnancy-associated plasma protein A; PlGF, placental growth factor; sCD40L, soluble CD40 ligand.

abnormal) in all patients, and between 3.3% and 38.6% in those receiving placebo (figures 2 and 3).

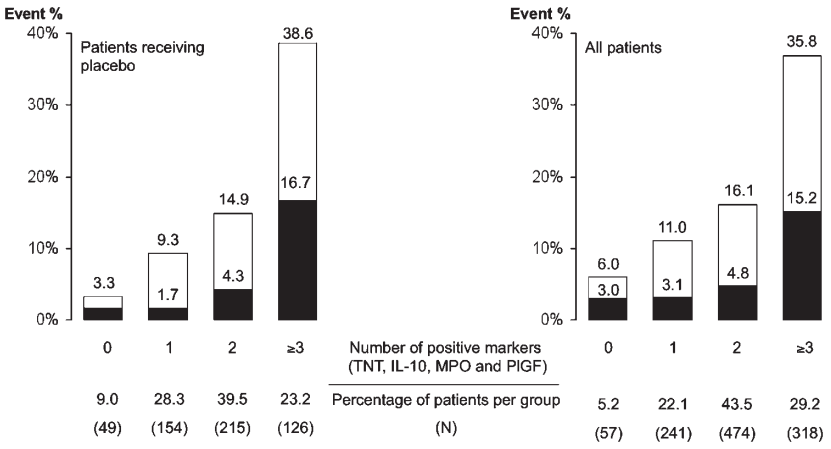

Figure 2. Multimarker risk score.
Multimarker risk score with separate risks for all-cause mortality (black squares) and non-fatal myocardial infarction (white squares) in patients treated with placebo (left panel) and in the entire study population (right panel) at 4 years of follow-up after counting the absence or presence of one, two, or three or more biomarkers above the threshold levels. The markers used are: troponin-T (TnT), interleukin 10 (IL-10), myeloperoxidase (MPO), placental growth factor (PlGF).

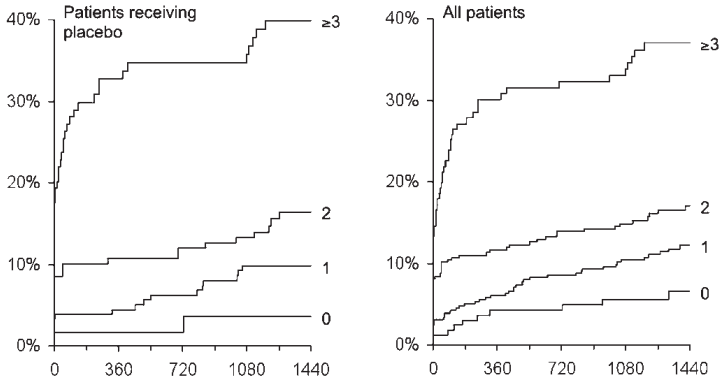

Figure 3. Kaplan–Meier analysis for the multimarker model.
Kaplan–Meier curves of all-cause mortality or non-fatal myocardial infarction during 4-year follow-up for patients treated with placebo (left panel) and the entire study population (right panel) with none, one, two, or three or more biomarkers above the threshold levels. The markers used here are: troponin-T, interleukin 10, myeloperoxidase and placental growth factor.

DISCUSSION

The present study is the first to report a long-term post-ACS multimarker risk prediction model for which biomarkers are selected on the basis of adjustment for baseline clinical patient characteristics as well as adjustment for all other analysed biomarkers. The results add to the growing body of evidence that novel biomarkers reflecting atherosclerotic burden or disease activity independently predict the long-term risk of death and non-fatal infarction in patients with an ACS. Elevated baseline levels of placental growth factor, myeloperoxidase and low levels of the anti-inflammatory cytokine IL-10 were independently associated with adverse long-term outcomes in patients with non-ST-segment elevation acute coronary syndrome (NSTEACS). These findings support the pathophysiological concept of a chronic (vascular) inflammatory basis of atherosclerosis,[16-18] with an acute superimposed process in the setting of an ACS.[1] The present data indirectly suggest that elevated biomarkers at baseline reflect a chronic inflammatory process in the coronary vessel wall that may indeed result in repetitive occurrences of cardiovascular events during long-term follow-up. The predictive value of the selected inflammatory markers was independent of baseline clinical patient characteristics and index diagnosis experienced during the initial incident (correction for unstable angina or non ST-elevation MI took place by adding TnT as a covariate in all our multivariate analysis). Moreover, multivariate analysis in a model including all biomarkers and baseline clinical patient characteristics proved that four out of seven biomarkers, but only two out of 14 baseline clinical patient characteristics (as described in table 2) remained significantly associated with the endpoint. The incremental value of novel biomarkers as risk predictors over traditional patient characteristics was previously described in another large cohort of non ST-elevation ACS patients in the GUSTO IV trial.[12] Our data might suggest that biomarkers improve risk prediction through their proposed capability to reflect disease biology, instead of mere patient characteristics. Accordingly, a combination of multiple biomarkers, which reflect different pathophysiological components of CAD, might aggregate risk prediction properties. The risk of death or non-fatal MI was calculated in this same line of thought using a simple risk stratification model by counting the number of markers outside the normal range. We investigated the role of myocardial necrosis (TnT) together with oxidative stress (myeloperoxidase), and chronic background vascular inflammation (PlGF and IL-10) for the development of a future cardiovascular event. As there was indeed an important increase in risk if patients showed an increasing number of abnormal biomarker values (figures 2 and 3), this simple stratification might aid to adjust and intensify treatment in such patients with a detrimental biomarker profile. A more aggressive therapeutic strategy, for instance, might prove to be useful not only in treating the current acute event, but also to prevent later events. In the future, this strategy might even include a more specific anti-inflammatory treatment

such as blocking of the PlGF receptor or reducing the activity of circulating PlGF levels by the administration of soluble vascular endothelial growth factor receptor 1.[19]

Multimarker strategies also provide a window of opportunity for the selection of candidate biomarkers for risk stratification. Previous studies of the same patient cohort reported that, after multivariate adjustment only for baseline clinical patient variables, hsCRP,[2] sCD40L[11] and PAPP-A[5] remained independent risk predictors of adverse cardiac events at 6 months follow-up. In this 4-year analysis, however, we observed that hsCRP, sCD40L and PAPP-A were significant predictors in univariate analysis, but lost significance after correction for other biomarkers, suggesting that the remaining biomarkers might be better post-ACS predictors for long-term risk. This is remarkable, particularly for hsCRP as this marker has been the focus of extensive research and has been suggested as the most likely candidate for clinical application.[20,21] Obviously, the selection of valuable candidate biomarkers for risk prediction of future coronary events on the basis of a single biomarker as well as in the setting of a multimarker model requires further elucidation. Preferably, future research will also clarify the appropriate cut-off values and the actual prediction windows of novel biomarkers across different patient groups.

Previous studies have shown an interaction between allocated treatment and levels of biomarkers for short-term follow-up.[11-15] Although formal testing did not show a significant interaction between the biomarkers and treatment with abciximab with respect to long-term event rates, we performed separate analyses for the placebo group and the entire study population. As the obtained data consistently indicate that TnT, IL-10, myeloperoxidase and PlGF are independent predictors for long-term outcome, we conclude that our findings have general applicability if confirmed in other trials including more heterogeneous study populations of patients with atherosclerosis and ischaemic coronary syndromes.

We acknowledge that this investigation has some limitations. First, the long-term follow-up data that were obtained from multiple sources were not adjudicated by an independent clinical event committee. The applied criteria for MI might thus have differed between investigators, and some events may actually have been missed. Second, no information is available on longterm medical treatment such as statin therapy or ACE inhibition[22,23] with their suggested anti-inflammatory effects, which might have influenced patient outcomes. However, it is unlikely that this has resulted in a differential bias between patients with or without elevated biomarker levels. In this respect, it should be emphasised that the investigators who collected long-term follow-up data (EB, TL) were blinded for any information on baseline data (including biomarker levels).

When using such a simple risk model with dichotomised biomarker data, quantitative information might be lost as higher levels for one or another biomarker could correspond with different individual risk. However, by using simple cut-off values, physicians might be able to calculate the patients' risk more easily. With the help of this model in conjunction with the careful selection of other biomarkers and clinical variables, we

might be able to provide tailored treatment for the individual patient not only at the time of hospitalisation but also following discharge. The threshold levels of the markers are based on exploratory analyses illustrated in previous publications. Naturally, if possible, prospective validation should be performed. Finally, it remains to be determined whether assessment of these biomarkers at discharge, or at even later time points in stabilised patients, might demonstrate an even closer relationship between abnormal biomarker levels and the risk of future cardiovascular events.

Certain biomarkers have specific evolutionary patterns during admission for ACS[24] and thus the role of the exact timing of the biomarker measurements should be emphasised. In our study, blood samples were drawn at admission, on average 9 h after the last episode of angina. Its results should therefore not be extrapolated to biomarker measurements at other time points, for example, at discharge, when the same biomarkers (at the same cut-off levels) theoretically might demonstrate a different predictive value. Although new troponin elevations are seldom found after the first 6-9 h,[25] the possibility exists that some patients who were classified 'troponin-negative' in fact would prove to have elevated levels of circulating troponin in case of measurement at later time points. As troponin elevations are related to a worse prognosis, this type of potential unidirectional misclassification might have led to an underestimation of the relation between troponin elevation and adverse outcomes in our analyses.

CONCLUSION

In patients with NSTEACS, biomarkers reflecting distinct aspects of the underlying atherosclerotic process, and myocardial damage of the initial cardiac event can assist in predicting longterm adverse cardiac outcomes. The use of combinations of selected biomarkers adds incremental predictive value to further risk stratification in an otherwise seemingly homogeneous NSTEACS population.

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Competing interests None.

Ethics approval This is a biomarker analysis in samples that derive from a multicentre randomised controlled trial for which ethics committee approval was obtained at both the national and local hospital levels.

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CORONARY VULNERABILITY

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3

LIPOPROTEIN(A), INTERLEUKIN-10, C-REACTIVE PROTEIN, AND 8-YEAR OUTCOME AFTER PERCUTANEOUS CORONARY INTERVENTION

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ABSTRACT

Background: This prospective study investigated the association between preprocedural biomarker levels and incident major adverse cardiac events (MACE) in complex patients undergoing percutaneous coronary intervention (PCI) with sirolimus-eluting stenting.

Hypothesis: Lipoprotein(a) (Lp[a]), interleukin-10 (IL-10), and high-sensitivity C-reactive protein (CRP) have long-term prognostic value in patients undergoing PCI.

Methods: Between April 2002 and February 2003, 161 patients were included in the study. Blood was drawn before the procedure, and biomarkers were measured. Patients were followed-up for MACE (death, nonfatal myocardial infarction, and repeat revascularization). Cox proportional hazard models were used to determine risk of MACE for tertiles of biomarkers. Both 1-year and long-term follow-up (median, 6 years; maximum, 8 years) were evaluated.

Results: Mean age was 59 years, and 68% were men. During long-term follow-up, 72 MACE occurred (overall crude cumulative incidence: 45% [95% confidence interval (CI): 37%-52%]). Lp(a) was associated with a higher 1-year risk of MACE, with an adjusted hazard ratio (HR) of 3.1 (95% CI: 1.1-8.6) for the highest vs the lowest tertile. This association weakened and lost significance with long-term follow-up. IL-10 showed a tendency toward an association with MACE. The 1-year HR was 2.1 (95% CI: 0.92-5.0). Long-term follow-up rendered a similar result. The association of CRP with MACE did not reach statistical significance at 1-year follow-up. However, CRP was associated with long-term risk of MACE, with an HR of 1.9 (95% CI: 1.0-3.5).

Conclusions: In this prospective study, preprocedural Lp(a) level was associated with short-term prognosis after PCI. The preprocedural CRP level was associated with long-term prognosis after PCI.

INTRODUCTION

Coronary artery disease (CAD) remains a leading cause of morbidity and mortality in the Western world. Percutaneous coronary intervention (PCI) has significantly reduced consequences of CAD.[1] Nevertheless, post-PCI patients still constitute a high-risk group for recurrent events and cardiovascular mortality. To improve long-term prognosis in post-PCI patients, first and foremost, further enhancement of risk stratification is needed. Identification of high-risk patients may then serve as a guide to apply or withhold more aggressive treatment.

Biomarkers have received much attention as predictors of CAD in the past decade. Several biomarkers have been associated with incident coronary events, both in the general population and in patients with known CAD.[2,3] Furthermore, a body of research is growing on emerging biomarkers.[4] Data on the association between preprocedural biomarker levels and prognosis after PCI are less elaborate.

In the current study, which was conceived nearly a decade ago, we postulated that early preprocedural markers may predict later cardiac events. At the time of study commencement, several lines of evidence had already confirmed that inflammation plays a major role in the pathogenesis of atherosclerotic lesions of vascular walls. C-reactive protein (CRP) was strongly implicated[5] and was considered a promising candidate for the present study. Furthermore, attention had also been directed toward interleukin (IL)-10 as 1 of the most important mediators that physiologically limits and downregulates inflammation.[6] With regard to lipid biomarkers, although evidence was not always consistent, lipoprotein(a) (Lp [a]) was deemed a promising novel biomarker.[5] Therefore, we have investigated the long-term prognostic value of these 3 biomarkers in complex patients undergoing PCI with sirolimus eluting stenting.

Apart from assessing prognostic value at 1 year of follow-up, we also examined the value at a maximum follow-up of 8 years. Such extensive follow-up data are currently scarce and enabled us to examine the patterns of the associations between biomarkers and cardiac events over time.

METHODS

Patient Population and Baseline Data Collection

The study population consisted of a subset (n = 161) of the RESEARCH registry (Rapamycin-Eluting Stent Evaluated At Rotterdam Cardiology Hospital), that has been described elsewhere.[7] Briefly, RESEARCH is a single-center registry conducted with the main purpose of evaluating the safety and efficacy of sirolimus-eluting stent (SES) implantation for patients treated in daily practice. To include a patient population representative

of the real world, from April 2002 onward a policy was adopted of using SES (Cypher; Johnson & Johnson-Cordis, Cordis Europa NV, Roden, The Netherlands) as the default strategy for every PCI. The general indications for PCI included stable angina pectoris, unstable angina pectoris, and acute myocardial infarction (MI). For the current study, patients defined as complex undergoing PCI between April 2002 and February 2003 were included. These complex patients typically had SES implanted in bifurcations, left main coronary, chronic total occlusions, very small vessels, and long stented length (>36 mm). Patients were included during office hours, with the exception of random periods of absence of the responsible investigator. The study was approved by the hospital ethics committee and is in accordance with the Declaration of Helsinki. Written informed consent was obtained from every patient.

Baseline characteristics were assessed by screening medical records at the time of the procedure and included demographics, medical history, medication use, and cardiovascular risk factors. Information on cardiovascular risk factors included smoking, diabetes mellitus, hypertension, and hypercholesterolemia as diagnosed and registered in the medical records by treating physicians.

Biomarker Measurements

Blood was drawn immediately before PCI. High-sensitivity CRP (hsCRP) was determined directly at the Clinical Chemistry Department of Erasmus Medical Center by using Rate Near Infrared Particle Immunoassay (Immage Immunochemistry System; Beckman Coulter, Inc., Brea, CA). This system measures concentrations from 0.2 to 1440 mg/L, with a within-run precision <5% and a total precision <7.5%.

Subsequently, blood samples were stored at −80°C at Erasmus Medical Center, after which they were transported on dry ice to Dunedin, New Zealand, where IL-10 and lipoprotein (a) (Lp [a]) were measured. IL-10 was measured using the Quantikine HS immunoassay (HS100B) (R&DSystems, Minneapolis, MN). The minimal detectable dose of this assay is 0.5 pg/mL, the intra-assay precision is <8.5%, and the interassay precision is <15.6%. Lp(a) was measured using a double sandwich enzyme-linked immunosorbent assay as previously described.[8] This assay detects all apolipoprotein (a) isoforms on an equivalent molar basis and is considered the most accurate method for Lp(a) measurement.[9] This assay measures concentrations from 2 to 600 nmol/L, with coefficients of variation <10%.

End Point Definitions

The primary outcome was the occurrence of major adverse cardiac events (MACE), defined as: 1) death, 2) nonfatal MI, or 3) repeat revascularization. MI was defined as a diagnosis made by a cardiologist based on the combination of typical ischemic chest complaints and objective evidence of myocardial necrosis as demonstrated by the elec-

trocardiogram or elevated cardiac markers. Revascularization was defined as a repeat intervention (surgical or percutaneous) to treat a luminal stenosis in any epicardial vessel.

Follow-up

Information about in-hospital outcomes was obtained from an electronic clinical database for patients maintained at Erasmus Medical Center, Rotterdam and by review of hospital records for those discharged to referring hospitals. Postdischarge survival status was obtained from municipal civil registries. Postdischarge repeat interventions and rehospitalizations were prospectively assessed during follow-up. Yearly questionnaires with information about anginal status and medication use were sent to all living patients, and treating physicians and institutions were contacted whenever necessary for additional information.

Statistical Analysis

We calculated means and proportions of the baseline characteristics and crude cumulative incidence. Subsequently, we divided biomarker levels into categories (tertiles and detectable vs nondetectable when appropriate) and calculated means and proportions of baseline characteristic according to these categories. To test for trends, we used linear regression for continuous variables, logistic regression for dichotomous variables, and multinomial regression for variables with more than 2 categories. We used ln-transformed continuous levels of biomarkers as the independent variable.

To address the predictive value of baseline biomarker levels, we calculated relative risks of MACE associated with increasing tertiles of CRP (cutpoints 1.9 and 4.5 mg/L), Lp(a) (cutpoints 9.8 and 65.2 nmol/L), and IL-10 (cutpoints 0.4 and 3.2 ng/mL) by Cox proportional hazards analysis. The proportional hazards assumption was tested by drawing log minus log plots of the survival function, which confirmed that the assumption was met. We adjusted for age and sex (model 1), and subsequently for age, sex, smoking, diabetes mellitus, hypertension, and hypercholesterolemia (model 2). First, we truncated follow-up time at 1 year. Subsequently, we examined very long-term outcome by taking complete follow-up into account. We repeated all analyses using ln-transformed continuous values of the biomarker levels instead of tertiles to demonstrate trends.

Values for covariates were missing in <1% of the patients, except for previous MI (missing in <2%). Given these low percentages, we chose to perform a complete-case analysis. All analyses were conducted with SPSS 17.0 for Windows (IBM, Armonk, NY). All tests were 2-sided.

RESULTS

The mean age of the patients was 59 years, and 68% were male (Table 1). The median follow-up was 6 years, with a maximum of 8 years. The total number of person-years of follow-up amounted to 709 years. A total of 39 and 72 MACE occurred after 1 year and 8 years of follow-up, respectively. The overall crude cumulative incidence was 45% (95% confidence interval [CI]: 37%-52%).Occurrence of MACE was highest at the beginning of the follow-up period and subsequently declined. Crude cumulative incidence over the first year of follow-up was 24% (95% CI: 18%-31%).

The median (interquartile range) levels of biomarkers were 3.03 (1.33–5.72) mg/L for CRP, 20.8 (6.08–84.9) nmol/L for Lp(a), and 1.81 (0–4.48) ng/mL for IL-10, respectively. All biomarker distributions were right-skewed. CRP was available in all patients and

Table 1. Baseline Characteristics of the Study Population (n = 161)

Variable	
Age, y	59.4 ± 11.3
Men	110 (68%)
Hypertension	63 (39%)
Hypercholesterolemia	125 (78%)
Diabetes mellitus	31 (19%)
Current smoking	51 (32%)
History of myocardial infarction	53 (34%)
History of CABG	18 (11%)
History of PCI	43 (27%)
Family history of coronary disease	60 (38%)
Clinical presentation	
Stable angina	85 (54%)
Unstable angina	45 (28%)
Acute myocardial infarction	29 (18%)
No. of diseased vessels	
1 vessel	70 (44%)
2 vessels	51 (32%)
3 vessels	38 (24%)
C-reactive protein, mg/L	3.03 (1.33–5.72)
Lipoprotein (a), nmol/L	20.8 (6.08–84.9)
Interleukin-10, ng/mL	1.81 (0.00–4.48)

Abbreviations: CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention. Categorical variables are expressed as number (percentage). Valid percentages are reported.Values of continuous variables are expressed as mean ± standard deviation or as median (interquartile range) in case of skewed distribution.

below the detection limit in 4 patients (2.5%). For trend analysis, the data points in these patients were imputed by dividing the lowest detectable limit of the assay (0.2 mg/L) by 2. Lp(a) measurement was missing in 3 patients and below the detection limit in 16 patients (10%). The lower limit of detection (2 nmol/L), divided by 2, was imputed in these patients for trend analysis. IL-10 measurement was missing in 3 patients and below the detection limit in 49 patients (31%). Because of this high percentage of undetectable levels, values were not imputed, and the *P* for trend was not computed. IL-10 was thus only examined in tertiles and as a dichotomous variable (detectable vs not detectable).

Table 2 displays baseline characteristics according to categories of Lp(a), CRP, and IL-10 level. CRP showed a positive association with diabetes mellitus ($P = 0.004$) and with number of diseased vessels ($P = 0.04$).

Table 3 shows hazard ratios (HRs) for MACE at 1 year of follow-up, where as Table 4 shows those at maximum follow-up. Lp(a) was associated with 1-year risk of MACE, with an HR of 3.1 (95% CI: 1.1-8.6) for the highest vs the lowest tertile. This association weakened and lost significance with long-term follow-up, the HR becoming 1.6 (95% CI: 0.86 – 3.1). IL-10 showed a tendency toward an association with MACE. The 1-year HR was 2.1 (95% CI: 0.92 – 5.0) for the highest vs the lowest tertile. Long-term follow-up rendered a similar result, the HR becoming 1.7 (95% CI: 0.94 – 3.2). The association of CRP with MACE did not reach statistical significance at 1-year follow-up and was 1.7 (95% CI: 0.77 – 3.8). However, CRP was associated with long-term risk of MACE, with a HR of 1.9 (95% CI: 1.0 – 3.5) for the highest vs the lowest tertile. The Figure 1 further illustrates these associations.

DISCUSSION

In this prospective study, we examined the association between preprocedural biomarker levels and incident MACE in patients undergoing PCI with sirolimus-eluting stenting. Lp(a) level was associated with MACE at 1-year follow-up. CRP level was associated with MACE at 8 years of follow-up. IL-10 showed a tendency toward an association with MACE.

Strengths of the current study include the collection of preprocedural biomarker levels, the availability of extensive long-term follow-up, and the measurement of multiple biomarkers. Furthermore, the study population consisted of complex patients, which generally suffer a high event rate as evidenced by the high incidence rate in this study. This enriched population may especially benefit from additional measures for risk stratification, enabling application or withholding of more aggressive treatment. However, at the same time, our focus on this selected patient group limits the generalizability of our findings to a broader PCI population.

Table 2. Baseline Characteristics According to Categories of Lipoprotein (a), Interleukin-10, and C-Reactive Protein Level

Baseline Characteristic	Lp (a), Tertiles				Il-10, Detectable vs Nondetectable			CRP, Tertiles			
	1 (n = 52)	2 (n = 54)	3 (n = 52)	*P* for Trend	Non-detectable (n = 49)	Detectable (n = 109)	*P* Value	1 (n = 53)	2 (n = 54)	3 (n = 53)	*P* for Trend
Age, y	58.0 ± 11.9	60.0 ± 11.8	60.6 ± 9.9	0.20	60.1 ± 10.7	59.2 ± 11.5	0.61	57.4 ± 12.8	61.6 ± 10.4	59.4 ± 10.1	0.15
Men	73	70	62	0.43	61	72	0.63	68	69	68	0.71
Hypertension	27	40	52	0.05	47	34	0.13	34	38	47	0.08
Hypercholesterolemia	71	77	85	0.28	80	77	0.70	81	77	76	0.31
Diabetes mellitus	19	17	23	0.87	27	17	0.15	9	19	30	0.004
Current smoking	44	25	25	0.14	37	29	0.32	30	25	40	0.78
History of MI	28	42	33	0.99	29	36	0.40	23	38	41	0.11
History of CABG	8	11	15	0.28	8	13	0.39	8	11	15	0.22
History of PCI	19	26	37	0.15	31	25	0.46	28	26	26	0.71
Family history of coronary disease	29	45	35	0.36	43	35	0.36	38	26	47	0.24
Number of diseased vessels				0.55[a]			0.75[a]				0.04[a]
1	52	42	37	Reference	47	44	Reference	53	42	36	Reference
2	29	30	39	0.31[b]	33	31	0.98[b]	30	30	36	0.27[b]
3	19	28	23	0.44[b]	20	25	0.48[b]	17	26	28	0.02[b]

Abbreviations: CABG, coronary artery bypass graft; CRP, C-reactive protein; Il-10, interleukin-10; Lp (a), lipoprotein (a); MI, myocardial infraction; PCI, percutaneous coronary intervention.

Categorical variables are expressed as percentage. Continuous variables are expressed as mean ± standard deviation. *P* for trend was obtained by linear, logistic, or multinomial regression, whichever was appropriate. [a]Overall *P* for trend. [b]*P* for trend for specific number of vessels.

Table 3. Hazard Ratios for Major Adverse Cardiac Events at 1 Year of Follow-Up

Biomarker	Cumulative Incidence (95% CI)	Hazard Ratio (95% CI)	
		MACE, Adjusted for Age and Sex	MACE, Multivariate Adjusted
Lipoprotein (a)			
Tertile 1	10% (2-18)	1.00 (reference)	1.00 (reference)
Tertile 2	31% (19-44)	3.7 (1.4-10.1)	3.3 (1.2-9.2)
Tertile 3	31% (18-43)	3.5 (1.3-9.6)	3.1 (1.1-8.6)
P for trend		0.04	0.06
Interleukin-10			
Tertile 1	17% (7-28)	1.00 (reference)	1.00 (reference)
Tertile 2	24% (13-35)	1.5 (0.63-3.5)	1.3 (0.52-3.3)
Tertile 3	29% (17-41)	2.1 (0.92-5.0)	2.1 (0.92-5.0)
Detectable vs nondetectable		1.6 (0.76-3.4)	1.5 (0.69-3.3)
C-reactive protein			
Tertile 1	21% (10-32)	1.00 (reference)	1.00 (reference)
Tertile 2	20% (10-31)	0.93 (0.40-2.2)	0.87 (0.37-2.1)
Tertile 3	32% (20-45)	1.6 (0.72-3.3)	1.7 (0.77-3.8)
P for trend		0.20	0.17

Abbreviations: CI, confidence interval; MACE, major adverse cardiac events (death, nonfatal myocardial infarction, and repeat revascularization).
Multivariate adjusted: adjusted for age, sex, smoking, diabetes mellitus, hypertension, and hypercholesterolemia.

Several other aspects of this study warrant consideration. Sample size was limited, which was primarily due to the relative novelty and complexity of the laboratory measurements at the time of study commencement in 2002. Nevertheless, our results on CRP, the marker that has been examined most elaborately in the past, confirm previous findings. As such, we expect that associations between other biomarkers and adverse events, if present, would be demonstrated in this cohort. We had a limited number of MACE available at 1-year follow-up (n = 39, 24%). This number of events suffices for the analyses adjusted for age and sex. However, we realize that use of a multivariate adjusted model for 1-year follow-up is not fully justified. In this regard, the choice to divide the sample into tertiles may also be debated. As such, we also performed the analyses using continuous biomarker levels to demonstrate trends. Nevertheless, using tertiles enables identification of a dose-response relationship. Lp(a) was below the detection limit in 10% of the patients and IL-10 in 30%. This is a commonly encountered challenge when measuring these types of biomarkers. An analysis based on tertiles circumvents this issue, and therefore we believe our findings are nevertheless informative.

Although we found an association of CRP, a sensitive marker of inflammation, with MACE at long-term follow-up of 6 years, the association was not significant at 1 year

Table 4. Hazard Ratios for Major Adverse Cardiac Events at 8 Years Follow-Up

Biomarker	Cumulative Incidence (95% CI)	Hazard Ratio (95% CI)	
		MACE, Adjusted for Age and Sex	MACE, Multivariate Adjusted
Lipoprotein (a)			
Tertile 1	33% (20-45)	1.00 (reference)	1.00 (reference)
Tertile 2	44% (31-58)	1.6 (0.86-3.0)	1.5 (0.79-2.9)
Tertile 3	56% (42-69)	1.9 (1.0-3.5)	1.6 (0.86-3.1)
P for trend		0.11	0.20
Interleukin-10			
Tertile 1	40% (27-54)	1.00 (reference)	1.00 (reference)
Tertile 2	44% (31-58)	1.2 (0.69-3.3)	1.2 (0.62-2.3)
Tertile 3	48% (34-62)	1.7 (0.95-3.2)	1.7 (0.94-3.2)
Detectable vs nondetectable		1.4 (0.83-2.4)	1.4 (0.78-2.4)
C-reactive protein			
Tertile 1	36% (23-49)	1.00 (reference)	1.00 (reference)
Tertile 2	39% (26-52)	0.98 (0.52-1.8)	0.93 (0.49-1.8)
Tertile 3	60% (47-74)	1.7 (0.98-3.1)	1.9 (1.0-3.5)
P for trend		0.016	0.009

Abbreviations: CI, confidence interval; MACE, major adverse cardiac events (death, nonfatal myocardial infarction, and repeat revascularization).
Multivariate adjusted: adjusted for age, sex, smoking, diabetes mellitus, hypertension, and hypercholesterolemia.

of follow-up. Because the point-estimate at 1-year follow-up was comparable to that at long-term follow-up, this is probably due to lack of statistical power. Levels of CRP have been elaborately investigated, and have been found to be associated with poorer cardiovascular outcomes in both healthy populations and patients with coronary artery disease.[3] Furthermore, several large studies have shown that higher preprocedural CRP levels are related to a greater long-term risk of adverse events after PCI.[10–12] These studies examined outcomes for up to 2 years of follow-up. Fewer data are available on long-term follow-up beyond 2 years. Gach et al examined the effect of hsCRP in stable patients undergoing PCI on adverse events during a mean follow-up of nearly 80 months, and found that increase in CRP after PCI was more predictive of MACE than CRP level before PCI.[13] However, this study was confined to 89 patients. Our results confirm association of preprocedural CRP levels with MACE during long-term follow-up, and expand these findings to a follow-up of 8 years.

Lp(a) showed an association with occurrence of MACE at 1-year follow-up, but the association lost statistical significance at 8-year follow-up. Lp(a) is a lipoprotein that may induce either a prothrombotic/antifibrinolytic effect, as apolipoprotein(a) resembles plasminogen but has no fibrinolytic activity, or may accelerate atherosclerosis, because

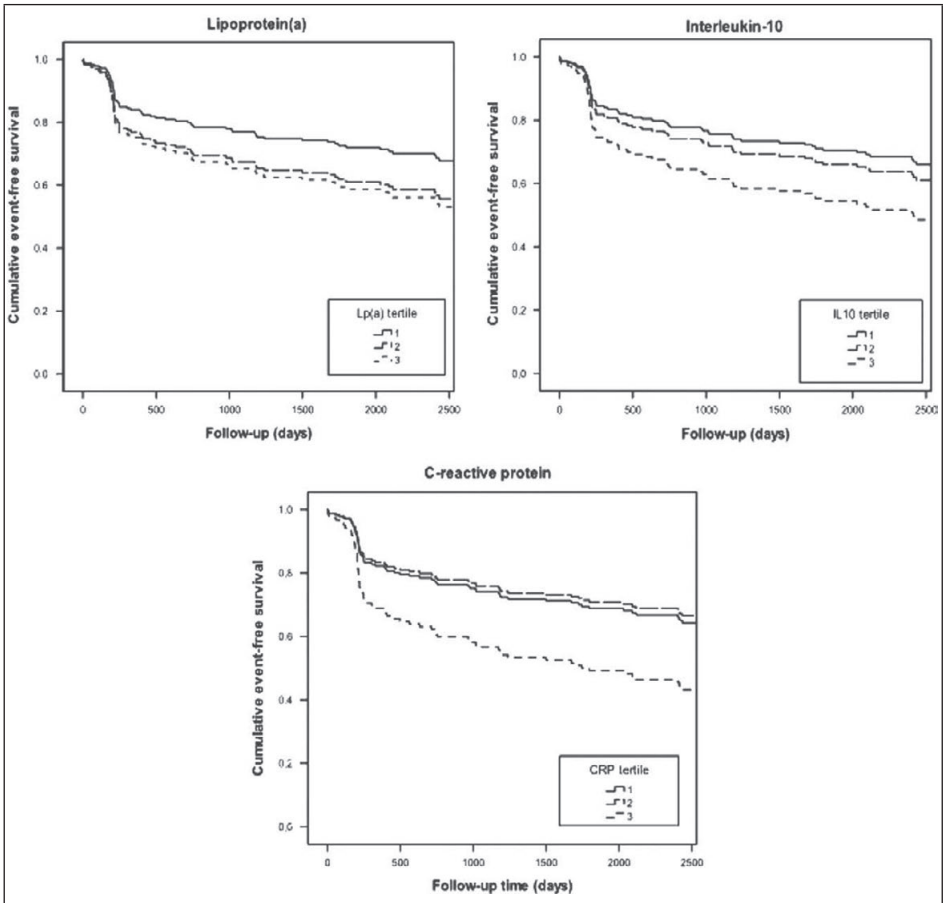

Figure 1. Multivariate-adjusted event-free survival until major adverse cardiac events.
All curves are adjusted for age, sex, smoking, diabetes mellitus, hypertension, and hypercholesterolemia. Abbreviations: CRP, C-reactive protein; IL 10, interleukin-10; Lp (a), lipoprotein (a).

like low-density lipoprotein, the Lp(a) particle is cholesterolrich, or both.[14] Consequently, numerous studies have been performed on the role of Lp(a) in restenosis, rendering inconsistent results.[15–21] Zairis et al investigated both in-stent restenosis and incidence of MACE in 483 consecutive patients with either stable or unstable coronary syndromes undergoing PCI with stenting.[22] Although they found no associations of Lp(a) with restenosis, they did find that high plasma levels of both CRP and Lp(a) were independently associated with MACE at long-term follow-up. They concluded that progression of atherosclerosis to a significant lesion in vessels not previously intervened on may play a significant role in the underlying pathophysiology as opposed to in-stent restenosis. The results of 1-year follow-up in the current study are in accordance with these findings. Several other studies have demonstrated positive associations of Lp(a) with adverse

events during long-term follow-up.[23,24] Our study confirms these results and provides additional information on very long-term follow-up, demonstrating loss of significance of the association in our (specific) patient population.

IL-10 displayed a tendency toward a positive association with long-term occurrence of MACE in the current study. IL-10 downregulates inflammatory activation of monocytes and macrophages by transcriptional and posttranscriptional inhibition of the entire range of proinflammatory cytokines.[25] The largest prospective study on the prognostic value of IL-10 levels in CAD examined 1090 patients with non–ST-segment elevation acute coronary syndrome during 4 years of follow-up for all-cause mortality and nonfatal MI, and found that a high IL-10 level is associated with better prognosis.[26] Other prospective studies in patients with known CAD mostly had follow-up time limited to several months and showed inconsistent results.[27–30] Data on IL-10 levels and restenosis after stenting are scarce, and results are also conflicting.[31,32] Further elucidation of the mechanisms involved and of the role of IL-10 herein is warranted to disclose the reasons behind these seemingly inconsistent findings.

CONCLUSION

In this prospective study of patients undergoing PCI, preprocedural Lp(a) level was associated with short-term prognosis, and preprocedural CRP level was associated with long-term prognosis. IL-10 showed a tendency toward an association with long-term prognosis. Large studies with extensive follow-up and simultaneous measurement of multiple biomarkers are needed to provide further insight into the role of biomarkers in risk stratification of patients undergoing PCI.

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CORONARY VULNERABILITY

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4

HIGH-SENSITIVITY C-REACTIVE PROTEIN PREDICTS 10-YEAR CARDIOVASCULAR OUTCOME AFTER PERCUTANEOUS CORONARY INTERVENTION

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ABSTRACT

Aims: This study aims to evaluate the prognostic value of high-sensitivity CRP (hsCRP) during 10-year follow-up after percutaneous coronary intervention (PCI).

Methods and results: Between April and October 2002, hsCRP was measured in 468 all-comer patients who underwent PCI with sirolimus-eluting stent implantation for stable coronary artery disease or acute coronary syndrome. Primary endpoint was the composite of all-cause mortality or myocardial infarction at 10-year follow-up. The Kaplan-Meier event curves displayed ongoing divergence of the hsCRP groups (hsCRP <1 mg/L: 14.7% vs. 1-3mg/L: 31.1% vs. >3mg/L: 43.1%). After adjustment for established cardiovascular risk factors and clinical presentation in a Cox regression model, higher CRP levels were associated with higher incidence of the composite endpoint (>3mg/L vs. <1 mg/L: HR 2.87, 95%CI 1.69-4.87, p<0.001; 1-3mg/L vs. <1mg/L: HR 2.30, 95%CI 1.31-4.03, p=0.004). Although adding hsCRP to a prediction model containing conventional cardiovascular risk factors did not significantly improve discriminatory power (area under the receiver operating characteristic curve 0.71 to 0.73, p=0.56), hsCRP was able to improve risk classification (net reclassification index=0.40, p=<0.001).

Conclusions: In patients undergoing PCI, higher CRP levels at the time of the procedure are predictive for 10-year mortality and myocardial infarction. HsCRP may be an useful biomarker to further improve risk assessment in patients undergoing PCI.

INTRODUCTION

Chronic inflammation is considered to be a essential component in the pathogenesis and progression of atherosclerosis.[1-5] Increasing amounts of data suggest a possible role for C-reactive Protein (CRP) at different stages of atherogenesis and the atherosclerotic process.[6] C-reactive Protein (CRP), member of the pentraxin family of innate immune response proteins, is produced in the liver in response to various cytokines, such as Interleukin-6, Interleukin-1β and Tumor Necrosis Factor-α.[7] The precise pathophysiological role of CRP in the instigation and progression of atherosclerosis remains unclear. Still this lack of current basic pathophysiological insight detracts little from the accumulating evidence indicating an association between elevated CRP levels and adverse outcome in CAD patients undergoing percutaneous coronary intervention (PCI). CRP is associated with an increased incidence of cardiac events, including all-cause and/or cardiovascular mortality, (non-fatal) acute myocardial infarction and (urgent) revascularization in multiple studies.[8-13] The majority of these results, however, derive from an era in which percutaneous revascularization took place by plain balloon angioplasty or bare metal stent implantation. Less is known about the predictive value after drug-eluting stent implantation or about long-term follow-up. This study aims to evaluate the prognostic value of high-sensitivity CRP (hsCRP) during 10-year follow-up after (PCI) in the drug-eluting stent era.

METHODS

Study population

The design of the Rapamycin-Eluting Stent Evaluated At Rotterdam Cardiology Hospital (RESEARCH) registry has been described in detail elsewhere.[14] RESEARCH is a single-center all-comers registry conducted with the main purpose of evaluating the safety and efficacy of sirolimus-eluting stent (SES, Cypher; Johnson & Johnson-Cordis, Cordis Europa NV, Roden, The Netherlands) implantation. In brief, SES implantation has been used as the default strategy for all consecutive percutaneous coronary interventions between April 2002 and February 2003 in the Erasmus MC, Rotterdam, the Netherlands. High-sensitivity CRP was prospectively measured in a subset of 468 consecutive RESEARCH patients that were enrolled between April 2002 and October 2002.

Ethics

This is an observational study. Patients were not subject to acts, neither was any mode of behavior imposed, otherwise than as part of their regular treatment. Therefore, this study was not subject to the Dutch Medical Research Involving Human Subjects Act, and written informed consent for a patient to be enrolled was not required. This study was

conducted according to the Privacy Policy of the Erasmus MC, according to the Erasmus MC regulations for the appropriate use of data in patient oriented research, and according to the Helsinki Declaration.

High sensitivity C-reactive protein

Serum samples were drawn immediately before the PCI procedure. High-sensitivity CRP (hsCRP) was determined at the Clinical Chemistry Department of Erasmus Medical Center by using Rate Near Infrared Particle Immunoassay (Immage Immunochemistry System; Beckman Coulter, Inc., Brea, CA). This system measures concentrations from 0.2 to 1440 mg/L, with a within-run precision <5% and a total precision <7.5%.

Clinical endpoints

As part of the RESEARCH registry, information about in-hospital outcomes was obtained from an electronic clinical database for patients maintained at our center and by review of hospital records for those discharged to referring hospitals. Postdischarge survival status was obtained from municipal civil registries. Yearly questionnaires were sent to all living patients to obtain information on anginal status and medication use. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information (i.e. discharge letters and coronary angiogram) whenever necessary.

The primary endpoint of this report was the composite of all-cause mortality or myocardial infarction at 10 years of follow-up. Myocardial infarction was defined as the clinical diagnosis of ST-segment elevation myocardial infarction (STEMI) or non-STEMI. The secondary endpoint was defined as all-cause mortality at 10 years of follow-up. All endpoints were adjudicated by trained personnel.

Statistical analysis

CRP levels were also categorized as low (<1 mg/L), intermediate (1-3 mg/L) or high (>3 mg/L) according to the recommendations from the Centers for Disease Control and Prevention and the American Heart Association.[15] Continuous variables were compared by analysis of variance (ANOVA) test and are presented as mean ± standard deviation or as median [interquartile range]. Categorical variables were compared by chi-square test and are presented in numbers and percentages. Patients lost to follow-up were considered at risk for death until the date of last contact, at which time-point they were censored. Cumulative event rates were estimated according to the Kaplan-Meier method. Kaplan-Meier event curves were compared by log-rank test. Cox proportional hazards regression analyses were performed to evaluate the associations between CRP and study endpoints. In multivariable analyses, the variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction,

clinical presentation and multivessel coronary disease were considered as potential confounders and were entered into the full model. The final results are presented as crude and adjusted hazard ratios (HR) with 95% confidence interval (95% CI). Receiver operating characteristic (ROC) curves were constructed to evaluate the supplemental value of these biomarkers for discrimination between cases and controls over conventional cardiovascular risk factors. The area under the ROC curves were compared using the method that was described by Hanley et al.[16] Additionally, continuous net reclassification improvement indices (NRI) were calculated to evaluate improvement in risk classification by the new biomarkers over conventional cardiovascular risk factors.[17] 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 patients was 61 ± 11 years and 69% were men (Table 1). Patients with diabetes (p=0.034), smokers (p=0.008) and patients with a history of myocardial infarction (p=0.001) had higher hsCRP levels (Table 2). Female patients tended to have higher CRP levels, although the difference compared to men was not statistically significant (p=0.074). Serum hsCRP concentrations were dependent on the clinical presentation (p<0.001). Patients with stable angina pectoris (median 2.0 [1.0-5.0] mg/L) had the lowest circulating CRP concentrations. Higher hsCRP levels were observed in patients with unstable angina pectoris (median 5.0 [2.0-11.0] mg/L) and patients with acute myocardial infarction (median 3.0 [1.0-6.0] mg/L) (p<0.001).

Incident events during follow-up

Vital status at 10-year follow-up was acquired for 464 (99.1%) patients. Response rate of the yearly questionnaires that were sent to all living patients was at least 79% in each year. After 10 years of follow-up, 146 patients reached the composite endpoint of all-cause mortality or myocardial infarction. The Kaplan-Meier event curves displayed ongoing divergence of the hsCRP groups (hsCRP <1 mg/L: 14.7% vs. 1-3mg/L: 31.1% vs. >3mg/L: 43.1%) (Figure 1).

Prediction of cardiovascular outcome

In univariable analysis, higher CRP levels were associated with a three-fold increased incidence of the composite endpoint of all-cause mortality or myocardial infarction during follow-up (high vs. low hsCRP: HR 3.54, 95%CI 2.14-5.88, p<0.001; 1-3 vs. <1 mg/L: HR 2.52, 95%CI 1.44-4.41, p=0.001) (Table 3). The association was observed in patients admitted

Table 1. Baseline characteristics

	TOTAL (n=468)	LOW CRP <1 (n=125)	INTERMEDIATE CRP 1-3 (n=128)	HIGH CRP >3 (n=215)	P
Patient characteristics					
Age, years	61.1 ± 11.1	59.4 ± 11.1	62.0 ± 11.0	61.5 ± 11.1	0.14
Men, n (%)	325 (69.4)	92 (73.6)	95 (74.2)	138 (64.2)	0.074
Diabetes mellitus, n (%)	57 (12.2)	9 (7.2)	13 (10.2)	35 (16.3)	0.034
Hypertension. n (%)	187 (40.0)	45 (36.0)	53 (41.4)	89 (41.4)	0.57
Hypercholesterolemia, n (%)	282 (60.3)	82 (65.6)	81 (63.3)	119 (55.3)	0.13
Smoking, n (%)	129 (27.6)	29 (23.2)	26 (20.3)	74 (34.4)	0.008
Previous MI, n (%)	158 (33.8)	32 (25.6)	35 (27.3)	91 (42.3)	0.001
Previous PCI, n (%)	122 (26.1)	29 (23.2)	32 (25.0)	61 (28.4)	0.55
Previous CABG, n (%)	46 (9.8)	9 (7.2)	14 (10.9)	23 (10.7)	0.51
High sensitivity CRP, mg/L	3.0 [1.0-7.0]				
Procedural characteristics					
Clinical presentation					<0.001
Stable angina pectoris, n (%)	224 (47.9)	72 (57.6)	75 (58.6)	77 (35.8)	
Unstable angina pectoris, n (%)	169 (36.1)	29 (23.2)	35 (27.3)	105 (48.8)	
Acute MI, n (%)	75 (16.0)	24 (19.2)	18 (14.1)	33 (15.3)	
Multivessel coronary disease, n (%)	273 (58.3)	65 (52.0)	79 (61.7)	129 (60.0)	0.23

Data are presented as mean ± standard deviation or as median [interquartile range].
CABG, coronary artery bypass grafting; CRP, C-reactive protein; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Table 2. Association between patient characteristics and circulating CRP concentration

	Median hsCRP [IQR]	P
Women	4.0 [2.0 – 9.0]	0.009
Men	3.0 [1.0 – 6.0]	
History of myocardial infarction	4.0 [2.0 – 8.0]	0.003
No prior myocardial infarction	3.0 [1.0 – 6.0]	
Diabetes Mellitus	4.0 [2.0 – 8.0]	0.035
Without Diabetes Mellitus	3.0 [1.0 – 7.0]	
Smokers	4.0 [2.0 – 7.5]	0.039
Non-smokers	3.0 [1.0 – 7.0]	
Clinical presentation		<0.001
Stable	2.0 [1.0 - 5.0]	
Unstable angina	5.0 [2.0 – 11.0]	
Acute myocardial infarction	3.0 [1.0 – 6.0]	

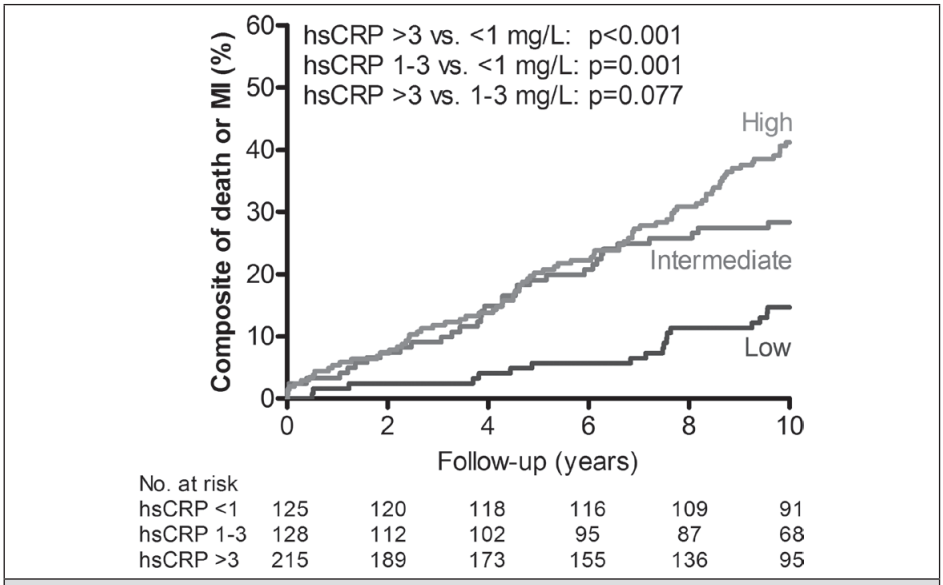

Figure 1. Prediction of cardiovascular outcome
CRP indicates C-reactive protein.

with ACS (high vs. low hsCRP: HR 3.64, 95%CI 1.74-7.61, p=0.001; 1-3 vs. <1 mg/L: HR 2.80, 95%CI 1.22-6.44, p=0.015) as well as in patients with stable angina (high vs. low hsCRP: HR 3.18, 95%CI 1.54-6.57, p=0.002; 1-3 vs. <1 mg/L: HR 2.33, 95%CI 1.10-4.95, p=0.028) (p for heterogeneity = 0.80). Higher CRP levels were also associated with all-cause mortality only (>3 vs. <1 mg/L: HR 3.64, 95%CI 2.05-6.44, p<0.001; 1-3 vs. <1 mg/L: HR 2.04, 95%CI 1.06-3.90, p=0.032). After adjustment for established cardiovascular risk factors and clinical presentation, CRP levels of >3 mg/L remained independently predictive for highest cardiovascular risk (HR 2.87, 95%CI 1.69-4.87, p<0.001), followed by CRP levels of 1-3 mg/L (HR 2.30, 95%CI 1.31-4.03, p=0.004) compared to CRP levels of <1 mg/L.

Table 3. Prediction of cardiovascular outcome

	Unadjusted HR (95%CI)	P	Adjusted* HR (95%CI)	P
Composite of all-cause mortality or myocardial infarction				
CRP 1-3 vs <1 mg/L	2.52 (1.44-4.41)	0.001	2.30 (1.31-4.03)	0.004
CRP >3 vs <1 mg/L	3.54 (2.14-5.88)	<0.001	2.87 (1.69-4.87)	<0.001
All-cause mortality				
CRP 1-3 vs <1 mg/L	2.04 (1.06-3.90)	0.032	1.81 (0.94-3.48)	0.075
CRP >3 vs <1 mg/L	3.64 (2.05-6.44)	<0.001	2.86 (1.57-5.22)	0.001

* Adjusted for age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction, clinical presentation and multivessel coronary disease.
CRP indicates C-reactive protein; HR, hazard ratio.

Discrimination

First, we evaluated a model for prediction of 10-year cardiovascular outcome that contained conventional cardiovascular risk factors, including age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction, clinical presentation and multivessel coronary disease. This model displayed an area under the ROC curve of 0.71 (95%CI 0.66-0.76) (Figure 2). Although not statistically significant, adding CRP to this model slightly improved discriminatory ability (area under the ROC curve = 0.73, 95%CI 0.69-0.78, p=0.56).

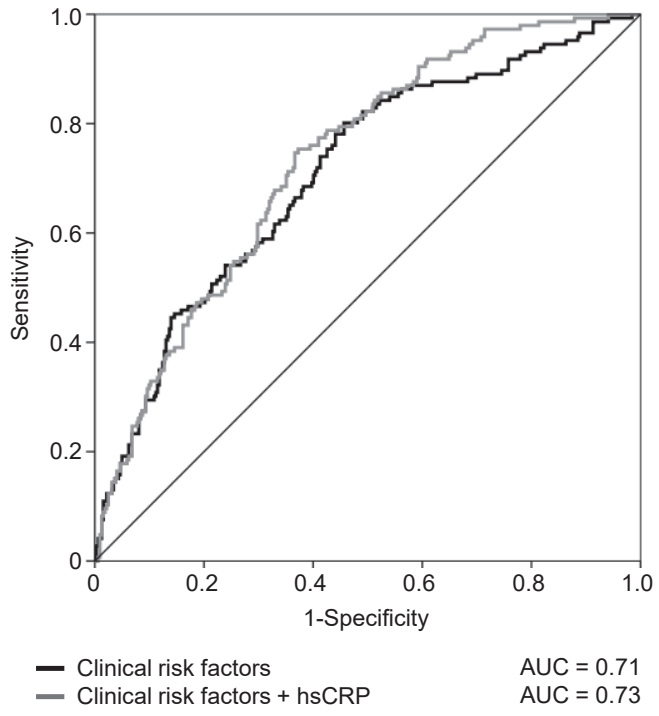

Figure 2. ROC curves displaying improved discrimination with C-reactive protein
Clinical risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction, clinical presentation and multivessel coronary disease.
AUC indicates area under the curve; CRP, C-reactive protein.

Reclassification

We examined whether adding CRP to the model consisting of conventional cardiovascular risk factors results in correct reclassification of risk of death or myocardial infarction during follow-up (Table 4). Baseline CRP level significantly improved the risk classification (NRI=0.40, 95%CI 0.20-0.60, p<0.001).

Table 4. Reclassification of predicted risk when adding C-reactive protein

	Predicted risk classified downward in new model*	Predicted risk classified upward in new model*	Total
Patients that reached primary endpoint, n (%)	22 (15.1)	124 (84.9)	146
Patients that remained event-free, n (%)	113 (35.1)	209 (64.9)	322

* New model includes clinical risk factors and CRP. Old model includes clinical risk factors only. Clinical risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction, clinical presentation and multivessel coronary disease.

DISCUSSION

This study investigated the association between circulating hsCRP concentration and 10-year cardiovascular outcome in patients undergoing PCI with drug-eluting stent implantation. The main finding is that a single baseline measurement of hsCRP is predictive for cardiovascular outcome with ongoing divergence of the survival curves until 10 years of follow-up. High hsCRP (>3 mg/L) levels were associated with a three-fold increased risk for mortality and the composite of mortality or myocardial infarction, while intermediate hsCRP (1-3 mg/L) were associated with a two-fold increased risk.

CRP is an acute phase protein and its concentration in serum reflects the inflammatory status of the patient.[18] Despite a lack of specificity for the cause of inflammation, many epidemiologic studies have shown significant associations between elevated serum CRP concentrations and the risk of recurrent cardiovascular events among patients with established coronary artery disease, and the incidence of first cardiovascular events among individuals with cardiovascular risk factors.[19-22] However, few studies are available on the prognostic value of hsCRP in patients undergoing PCI, while risk assessment at this certain time point is important and of particular interest in clinical practice. These studies have consistently showed that higher CRP levels measured at time of the PCI procedure for both acute coronary syndrome and stable CAD are predictive for an increased long-term risk of recurrent cardiovascular events and death. For example, Park et al. found that elevated CRP levels were significantly associated with increased risks of stent thrombosis, death, and MI during a median follow-up time of 3.9 years in patients receiving drug-eluting stents.[23] The longest reported follow-up period is 6 years.[21,24] To the best of our knowledge, this is the first study that extends the evidence on the predictive value of CRP to 10-years after PCI.

The mechanism underlying the association of CRP with prognosis may be two-fold.[25] Firstly, high CRP levels are previously shown to be associated with stent-thrombosis and restenosis after PCI with first generation drug-eluting stents.[15,23,25] A growing body of

evidence suggests that late adverse reactions to drug-eluting stents and bare-metal stents may be different in relation to pathogenesis, histopathologic features, and clinical presentation.[25] Although less evidence is available for second generation drug-eluting stents, Lasave et al. demonstrated that elevated CRP is also associated with neointimal hyperplasia in patients who received zotarolimus-eluting stent (a second generation drug-eluting stent) implantation.[26] Secondly, another underlying mechanism of the association of CRP with prognosis may be that high CRP levels are associated with coronary plaque burden and with new events in native vessels.[27]

Current clinical practice guidelines have indicated that measurement of hsCRP may be useful in 1. primary prevention, as an adjunct to other major risk factors to further assess absolute cardiovascular risk; and 2. in patients with stable coronary disease or acute coronary syndromes, as an independent marker for assessing the likelihood of recurrent events, including death, myocardial infarction, or restenosis after PCI.[28] For the latter indication, it should be noted that secondary preventive interventions with proven efficacy should not be dependent on hsCRP levels. Furthermore, the guidelines have stated that serial testing of hsCRP should not be used to monitor the effects of treatment. The results of our study confirm that hsCRP may be a useful biomarker to assess the risk of death and myocardial infarction in patients with established coronary artery disease who undergo PCI. Furthermore, we demonstrated that only a single measurement of hsCRP at the time of a PCI procedure is sufficient to provide information on cardiovascular risk for a period as long as 10 years. Therapeutic implications of increased inflammatory status after drug-eluting stent implantation are still under investigation.[25] Statins are shown to have anti-inflammatory properties.[29] Patients with intense activation of inflammatory cells, as detected by systemic CRP levels, are likely to enjoy the highest benefit from an high-dosed statin treatment.

Some limitations of this study need to be acknowledged. Firstly, this is a single center study. Caution is urged in extrapolating these results to other populations. However, other studies have showed consistent results on the long-term predictive value of hsCRP. Secondly, in this study, the prognostic value of hsCRP was evaluated in patients who underwent PCI with first generation drug-eluting stent implantation. Caution is urged in extrapolating these results to patients with new-generation drug-eluting stent implantation or patients with coronary artery disease in general. Thirdly, the number of patients at risk at the end of the follow-up period was relatively small. However, the 10-year association was strongly significant. Finally, despite using multivariable analysis to adjust for possible confounders that may be correlated to study outcomes, we cannot exclude the possibility of residual confounding. For example, in patients who presented with myocardial infarction, time-to-presentation was not registered in our study database. In these patients, CRP levels may be affected by on-going necrosis.

In conclusion, in patients undergoing PCI with drug-eluting stent implantation, high (>3 mg/L) and intermediate (1-3 mg/L) hsCRP levels are independently associated with a three-fold and two-fold increased risk, respectively, for mortality and myocardial infarction during follow-up. The survival curves of patients with high and intermediate hsCRP levels displayed ongoing divergence from that of patients with low hsCRP levels until 10 years after PCI, indicating that a single measurement of hsCRP at the time of a PCI procedure is sufficient to provide information on cardiovascular risk during a period as long as 10 years. Although adding hsCRP to a prediction model that contains conventional cardiovascular risk factors did not significantly improve discriminatory power, hsCRP was able to improve the risk classification over the conventional cardiovascular risk factors. Therefore, hsCRP may be an useful biomarker for long-term risk assessment in patients with established coronary artery disease and undergoing PCI.

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CORONARY VULNERABILITY

II

VULNERABLE PERIOD

CORONARY VULNERABILITY

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5

COHORT PROFILE OF BIOMARCS : BIOMARKER STUDY TO IDENTIFY THE ACUTE RISK OF A CORONARY SYNDROME, A PROSPECTIVE MULTICENTER BIOMARKER STUDY CONDUCTED IN THE NETHERLANDS

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ABSTRACT

Purpose: Progression of stable coronary artery disease (CAD) towards acute coronary syndrome (ACS) is a dynamic and heterogeneous process with many intertwined constituents, in which a plaque destabilising sequence could lead to ACS within short timeframes. Current CAD risk assessment models, however, are not designed to identify increased vulnerability for the occurrence of coronary events within a precise, short timeframe at the individual patient level. BIOMArCS was designed to evaluate whether repeated measurements of multiple biomarkers can predict such "vulnerable periods".

Participants: BIOMArCS is a multicentre, prospective, observational study of 844 patients presenting with ACS, either with or without ST-elevation and at least one additional cardiovascular risk factor.

Methods and analysis: We hypothesize that patterns of circulating biomarkers that reflect the various pathophysiological components of CAD, such as distorted lipid metabolism, vascular inflammation, endothelial dysfunction, increased thrombogenicity and ischemia, diverge in the days to weeks before a coronary event. Divergent biomarker patterns, identified by serial biomarker measurements during 1-year follow-up might then indicate 'vulnerable periods' during which CAD patients are at high short-term risk of developing an ACS. Venepuncture was performed every fortnight during the first half-year and monthly thereafter. As prespecified, patient enrolment was terminated after the primary endpoint of cardiovascular death or hospital admission for non-fatal ACS had occurred in 50 patients. A case-cohort design will explore differences in temporal patterns of circulating biomarkers prior to the repeat ACS.

Future plans and dissemination: Follow-up and event adjudication have been completed. Prespecified biomarker analyses are currently being performed and dissemination through peer-reviewed publications and conference presentations is expected from the third quarter of 2016.

Should identification of a 'vulnerable period' prove to be feasible, then future research could focus on event reduction through pharmacological or mechanical intervention during such periods of high risk for ACS.

Trial registration: NTR 1698 and NTR1106

INTRODUCTION

Generalized cardiovascular (CV) risk assessment models have proven to be valuable for longer term risk prediction in primary prevention settings, such as Framingham and SCORE [1,2], as well as in patients who experienced an acute coronary syndrome (ACS), such as the PURSUIT, TIMI and GRACE risk models. [3–5] Existing CV risk models largely depend on the presence and recognition of traditional risk factors and cardiovascular history complemented by biometric factors. Traditional CV risk factors, however, are absent in a significant part of the population that nevertheless develops CAD. [6] In contrast, the prevalence of traditional risk factors is also high among those fractions of the population that will never endure a CV event. [7]

According to the key philosophy behind existing CV risk prediction models, the individual patient is considered to be a member of a group that is exposed to a certain (low-intermediate-high) *constant* risk, whereas the incidence of acute CV events is considered a random process, with event probabilities directly related to that group risk. Consequently, CV risk models usually predict reasonably well on a *group* level, but only poorly outline the course of *individuals*. [8] In addition, current risk prediction models do not account for the *dynamic* nature of the atherosclerotic vascular wall of *individual* patients. Individual CAD patients actually do not have constant risks over time. [9] Long periods of stability, with minimal plaque progression and low risk of CV events are alternated by periods of increased plaque instability and rapid plaque progression [10], during which the risk of sudden plaque disruption, and thrombotic coronary occlusion within short time spans is high [11,12]. This is a complex and multifactorial pathophysiological process in which temporal variations in distorted lipid metabolism, vascular inflammation, endothelial dysfunction, increased thrombogenicity and myocardial ischemia play an important role. [9,11] Various established and novel serum biomarkers have been associated with each of these pathophysiological components, reflecting their presence and/or activity. [11,13–20] Furthermore, the biomarker's ability to fluctuate, at least in theory, perfectly suits monitoring short-term risks of a dynamic pathophysiologic process, as coronary artery disease. Integration of such dynamic information requires a conceptionally different perspective on risk prediction. Ideally, such a different approach might result in more precise and time-specific risk assessment for the occurrence of adverse cardiac events.

Therefore, we hypothesized that divergent biomarker patterns, detected through ambulatory and highly frequent blood sampling, could identify patients in a "vulnerable period" for the occurrence of an imminent myocardial infarction. In order to investigate this hypothesis, our aim is to obtain serial biomarker measurements as closely as possible prior to an ischemic event, yet in a phase in which the patient is still asymptomatic. Subsequent analysis of serial biomarker patterns up to the coronary event should elucidate

biomarker kinetics, patterns, appropriate cut-off values and prediction characteristics (such as timeframes), particularly shortly *prior* to the actual occurrence of an ACS.

COHORT DESCRIPTION

Study objectives

We designed the *BIOMarker study to identify the Acute risk of a Coronary Syndrome* (BIOMArCS) to evaluate whether biomarker patterns of (vascular) inflammation, distorted lipid metabolism, endothelial dysfunction, decreased endothelial regenerative capacity, increased thrombogenicity and ischemia diverge in days to weeks prior to an ACS. If our hypothesis is confirmed, then serial biomarker measurements might identify 'vulnerable periods' in the lifetime of patients with prevalent CAD, during which they are at increased risk of developing an ACS. Various hypothetically divergent biomarker patterns are depicted in Figure 1 (Panel A: divergence shortly prior to an ACS, Panel B: persistently higher (or lower) biomarker levels in the future cases, Panel C: higher intraindividual variability in the future cases).

Study cohort

BIOMArCS is a multi-centre, prospective, observational study conducted in 18 participating hospitals in the Netherlands. Patients who were admitted for an ACS, including unstable angina pectoris (UAP), non ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) with at least one additional CV risk factor were eligible for enrolment (Table 1). A total of 844 patients were enrolled from March 1st 2008 until January 26th 2015. Table 2 describes the baseline clinical characteristics of the enrolled cohort.

Blood samples were collected at admission, at the day of hospital discharge and subsequently every fortnight during the first six months after discharge, followed by monthly blood sample collection until 1 year. Patients were offered some flexibility in the follow-up scheme: visit windows are ±1 week, and a maximum of 2 consecutive visits are allowed to be skipped (for personal reasons). If logistic circumstances hindered inclusion during hospitalisation, patients could be included on the first outpatient visit within 6 weeks after discharge. The sample collection schedule was then adapted accordingly. Follow-up blood sampling was terminated permanently after coronary artery bypass grafting, hospital admission for heart failure, or a deterioration of renal function leading to a glomerular filtration rate <30 ml/min/1.73 m^2, in order to minimize bias in circulating biomarker concentrations. During the course of the study we observed prespecified discontinuation of biomarker sampling in 13 patients who were revascularized through CABG at a median follow-up duration of 116 days after the index-ACS. In these patients, samples were taken up until the bypass operation.

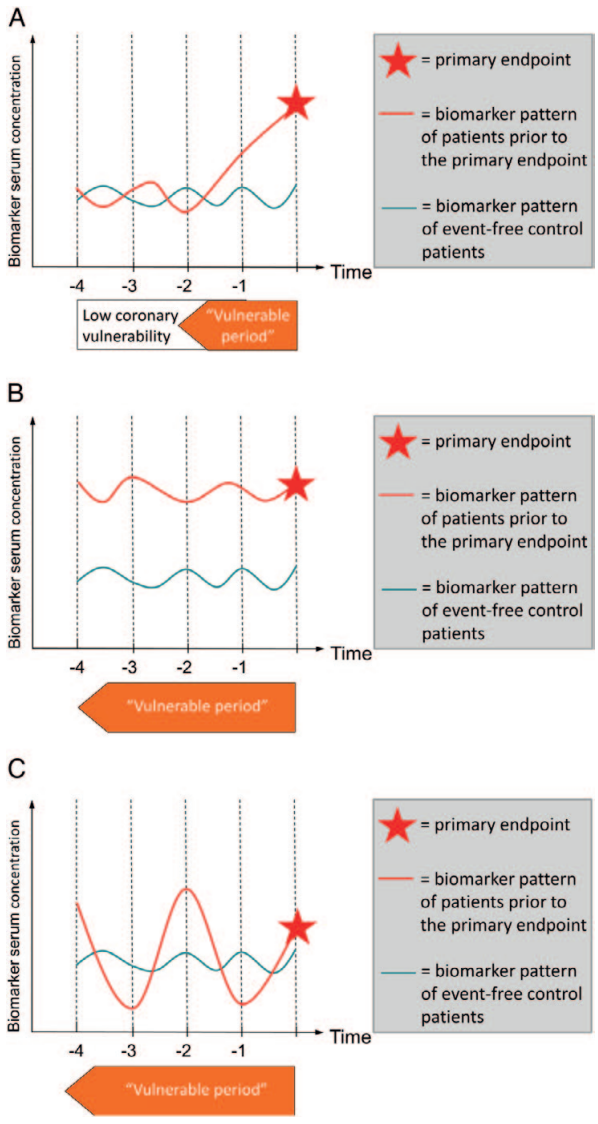

Figure 1. Different hypothetical scenarios of biomarker evolution during stable and vulnerable periods in the lifetime of a patient with coronary artery disease

Panel A describes a scenario in which biomarker patterns are relatively stable in a period of low coronary vulnerability, but are clearly divergent and upregulated shortly prior to the primary endpoint. Panel B describes a potential scenario in which the "vulnerable period" for a coronary event is relatively longer and characterized by persistently higher biomarker levels. Depending on the specific biomarker, this scenario could also apply in case of persistently lower (instead of higher) levels. Panel C depicts a divergent biomarker pattern in which a high degree of variability is associated with an increased risk of adverse cardiac outcome. Naturally, numerous variations and combinations of the above mentioned scenarios can be proposed for each specific biomarker depending on its characteristic pattern and kinetics.

Table 1. **Inclusion and exclusion criteria**	
Inclusion: a patient must meet all criteria	
1	Age ≥ 40 years
2	Complaints of typical ischemic chest pain, lasting 10 minutes or more within the preceding 24 hours prior to presentation
3a	ECG: (non-)persistent ST segment elevation >1.0 mm in two or more contiguous leads, or dynamic ST segment depression >1.0 mm in two or more contiguous leads, *OR*
3b	Biochemical evidence of myocardial injury: CK-MB or (high-sensitivity) Troponin I or (high-sensitivity) Troponin T elevation according to the applicable ESC guidelines of non ST-elevation acute coronary syndromes
4	Presence of at least 1 of the following risk factors: age ≥ 75 years, diabetes, prior cardiovascular disease, prior cerebrovascular disease and prior peripheral arterial disease. In addition, other risk factors mentioned below can be considered as well, but each only counts as half a risk factor, i.e. two of these are required for inclusion: age ≥ 65 years in men, age ≥ 70 years in females, hypertension, hypercholesterolemia, current smoking, or microalbuminuria†, positive family history of coronary artery disease ‡
5	Written informed consent
Exclusion: a patient cannot be included in case of any of the criteria below	
1	Myocardial ischemia precipitated by a condition other than atherosclerotic coronary artery disease
2	Left ventricular ejection fraction <30%, or end-stage congestive heart failure (NYHA class III or IV)
3	Renal dialysis, or severe chronic kidney disease with measured or calculated GFR (Cockroft-Gault or MDRD4 formula) of <30 ml/min/1.73 m^2
4	Co-existent condition with life-expectancy < 1 year or otherwise not expected to complete follow-up

GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; NYHA: New York Heart Association classification

† defined as >2.5-25 mg albumin/mmol creatinine for men and >3.5-35 mg for women, or >20-200 mg/l urinary albumin concentration in a single urine sample

‡ angina pectoris, myocardial infarction, or sudden abrupt death without obvious cause, before the age of 55 in a first-degree blood relative

A trained research nurse interviewed the patients at each visit and obtained data on anginal status (Canadian Cardiovascular Society classification), heart failure symptomatology (New York Heart Association classification), and specific factors that might influence biomarker levels, e.g. smoking, the occurrence of infections, inflammatory or allergic responses, alterations in medication, interventional or operative procedures and hospital admission.

This is an observational study. As such, it does not interfere with patient treatment. All patients were treated to prevailing guidelines and at the discretion of the investigator. The study protocol has been approved by the Institutional Review Board of all participating hospitals. Patients were only included after they provided written informed consent. The consent enables the investigators to enquire on the patients health status up to 15 years after enrolment.

Table 2. Baseline characteristics of the entire cohort of 844 patients	
Presentation and initial treatment	
Age, years	62.5 (54.3, 70.2)
Man	77.9
Admission diagnosis	
STEMI	51.7
NSTEMI	37.7
UAP	10.6
Culprit artery	
RCA	33.1
LM	2.5
LAD	31.9
LCX	16.5
Coronary angiography performed	94.4
Percutaneous coronary intervention	86.3
Maximum CK during admission (iU/L)	513 (200, 1370)
Cardiovascular risk factors	
Current smoking	40.5
Diabetes mellitus	23.5
Hypertension	55.5
Hypercholesterolemia	49.3
Cardiovascular history	
Prior percutaneous coronary intervention	26.2
Prior coronary artery bypass grafting	10.0
Prior myocardial infarction	26.9
Prior heart failure	2.4
Valvular heart disease	2.2
Prior stroke	9.0
Peripheral artery disease	8.9
Medication at first blood sample moment	
Aspirin	95.3
P2Y12 inhibitor	95.2
Vitamin K antagonist	6.8
Statin	96.2
Beta-blocker	89.8
ACE inhibitor or ARB	82.9

ACE: angiotensin converting enzyme; ARB: angiotensin II receptor blocker; CK: creatine kinase; LAD: left anterior descending artery; LCX: left circumflex artery; LM: left main coronary artery; MI: myocardial infarction; NSTEMI: non-ST-elevation myocardial infarcion; RCA: right coronary artery; STEMI: ST-elevation myocardial infarction; UAP: unstable angina pectoris

Continuous data are presented as median (25th, 75th percentile) values. Categorical data are presented as percentages. There are no missing data for any of the above mentioned variables.

Blood sample collection

Blood samples were first handled and securely stored on-site. After preparation, aliquots were frozen at −80 degrees Celsius within two hours after withdrawal. Long-term storage and biomarker analysis will take place at the department of Clinical Chemistry of the Erasmus MC. Apart from storage of serum, citrate- and EDTA-plasma, the BIOMArCS laboratory protocol also foresaw in collection and preservation of leukocytes for the purpose of genome analyses and flow-cytometric measurements of certain circulating leukocyte (monocyte) subsets that are thought to reflect endothelial regenerative capacity. [21]

Study endpoints

The primary endpoint is a composite of cardiac mortality or a clinical diagnosis of a non-fatal myocardial infarction or unplanned coronary revascularization due to progressive angina pectoris during 1-year follow-up. Any death will be considered cardiac unless documented to the contrary. Incident non-fatal myocardial infarction is defined as the combination of typical ischemic chest complaints and objective evidence of myocardial ischemia or myocardial necrosis as demonstrated by ECG and/or elevated cardiac markers. The criteria for non-fatal myocardial infarction during follow-up share the same definition as stated for the index event (points 1 and 2 of the study inclusion criteria). Study endpoints at 1-year follow-up were adjudicated by a Clinical Event Committee, which members were blinded for all biomarker data collected prior to the suspected incident event. At a later stage, events that occur after the first year and up to 15-years of follow-up (i.e. in the period without repeated blood sampling) will be adjudicated accordingly.

Sample size considerations

The incidence of the primary endpoint was estimated at 5% to 7%. Consequently, the number of patients who experience the primary endpoint ('cases') will be far less than those who remain endpoint-free. For reasons of efficiency, we will therefore apply the case-cohort design, [22] and temporal biomarker patterns of all cases will be compared with a limited number of non-cases.

For an adequate estimate of the required sample size, we applied 500 simulations of linear mixed-effects models for several scenarios (Table 3), which were based on repeated LDL-cholesterol (LDL-C) measurements from a pilot study with up to 5 measurements in 30 non-cases (non-published data). LDL-C was considered the dependent variable and endpoint-status the explanatory variable. We assumed that, on average, 6 to 10 repeated blood samples will be available in cases prior to the primary endpoint. Then, if 50 cases will be compared with 2 to 3 non-cases, a difference in the intercept of 0.17 to 0.21 mmol/l, and a difference in the slope of 0.06 to 0.11 mmol/l/month can be

Table 3. Results of simulations (500 for each scenario) to obtain an adequate estimate of the required sample size

Number of Cases	Number of non-cases	Number of repeated samples pp	Difference in intercept (mmol/l)	Difference in slope (mmol/l/month)
45	90	6	0.22	0.11
45	90	10	0.19	0.06
45	135	6	0.20	0.10
45	135	10	0.17	0.06
50	100	6	0.21	0.11
50	100	10	0.18	0.06
50	150	6	0.19	0.10
50	150	10	0.17	0.06
70	140	6	0.17	0.09
70	140	10	0.15	0.05
70	210	6	0.16	0.08
70	210	10	0.14	0.05

demonstrated between cases and non-cases with a power of 80% (2-sided test with an alpha error of 5%). We judged that these differences are small in clinical terms, and we considered the observed variations in LDL-C levels representative of changes in other biomarkers. In order to obtain 50 cases, given the anticipated incidence, a total of 700 to 1000 patients needed to be enrolled.

Construct of the case-cohort analysis set

A random, representative sample of 150 patients (random subcohort) will be chosen from all enrolled patients, and the patients who reach a study endpoint will be added. We anticipate that (50/1000)*150 = 8 to (50/700)*150 = 11 patients of the random subcohort will reach the primary endpoint. Hence, the expected ratio between patients with and without the primary study endpoint in the analysis set will be 1:2.8 to 1:2.9, which allows us to reveal clinically relevant differences in biomarker patterns with sufficient statistical power (see *Sample size considerations* above).

Biomarker selection and significance testing

Atherosclerosis and plaque destabilisation leading to intra-coronary thrombosis and an ACS is the result of a very heterogeneous process with many intertwined constituents. Vascular inflammation and endothelial disruption can result in thrombosis, which on its turn can exacerbate inflammation. [11] Many of the circulating biomarkers that have shown to adequately predict risks of future CV events, are therefore thought to reflect one or more of these distinct yet interdependent pathophysiological processes more

or less specifically. Currently, markers like those mentioned in Table 4 are considered to have high potential, and will be determined and reported in prespecified consecutive phases. Their selection is hypothesis-driven and based on current literature which is mainly based on one *single* measurement in time. [13–19,23–30] The development of biomarker levels shortly after presentation for ACS, and, more importantly, the frequently sampled biomarker patterns during the (asymptomatic) period preceding a subsequent event are unknown. A call for epidemiological research to establish the clinical value of serial analysis of biomarkers in atherosclerotic disease during long-term follow-up has repeatedly sounded,[12,31,32] but has not been answered as yet.

Table 4. Biomarker selection

The following biomarkers are considered of high-potential with regard to the BIOMArCS hypothesis and will be determined and reported in prespecified consecutive phases. Their selection is hypothesis-driven and based on current literature.

Phase 1

High sensitivity C-reactive protein (hs CRP)[1]
High sensitivity Troponin I (hsTnI)[1]
High sensitivity Troponin T (hsTnT)[2]
NT-pro BNP[3]
ST-2[4]
Creatinine[1]
Total cholesterol, HDL-Cholesterol[1], LDL-Cholesterol[5]

Phase 2 (in alphabetical order)

Copeptin
Ceramide (d18:1/16:0) as well as the following ceramide ratios:
- Cer(d18:1/16:0)/Cer(d18:1/24:0)
- Cer(d18:1/20:0)/Cer(d18:1/24:0)
- Cer(d18:1/24:1)/Cer(d18:1/24:0)

Cystatin-C
Galectin-3
Growth Differentiation Factor-15 (GDF-15)
Interleukins 1, 6, 8, 10, 18
Monokine Induced by interferon-Gamma (MIG)
Myeloperoxidase[3]
Placental growth factor (PlGF),
Plasminogen Activator Inhibitor 1 (PAI-1)
Pregnancy-associated plasma protein A (PAPP-A)
Regulated upon activation normal T cell expressed and secreted (RANTES)
Soluble CD40 ligand (sCD40L)
Tumor necrosis factor (TNF)
Von Willebrand Factor

Analyses of the markers in the first phase are to be performed on the following platforms/assays:

1) Coulter 5800 series, Beckman Coulter, Brea, California, USA
2) Cobas, Roche Diagnostics GmbH, Mannheim, Germany
3) custom built ELISA
4) Presage ST2 assay, Critical diagnostics, San Diego, California, USA
5) Friedewald Formula

Assays for the markers in the second phase have currently not been selected yet.

We will not limit our analyses to a selected number of markers. Biomarker research is a very rapidly evolving field in which novel and promising markers are regularly discovered. Exploratory analyses using proton nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry are also an option under consideration. [30]

We will perform several statistical tests to obtain significance levels for relations between biomarkers and study endpoints. For hypothesis-driven tests a two-tailed significance level of 0.05 will be used. For hypothesis-free tests corrections for inflation of the type I error due to multiple testing will be applied.

Etiologic and prognostic analyses of selected biomarkers

Compared to an analysis of the entire cohort, the advantage of a case-cohort design lies in its efficiency, whereas the ability to calculate absolute risks and rates is maintained. [22] We will perform etiologic as well as prognostic analyses. We utilize the framework of linear mixed-effects models to assess changes in biomarker levels over time, while accounting for the correlation between repeated follow-up measurements in each patient. [33] For both the fixed- and random-effects parts of the model we will test for possible nonlinear evolutions, which will be modelled by restricted cubic splines.

Biomarkers represent endogenous time-dependent covariate processes. We will therefore utilize the framework of joint models for longitudinal and survival data to investigate the relation between the serial biomarker measurements and the study endpoints. [34] Joint models combine the aforementioned linear mixed-effects models with a Cox regression model, adapted for a case-cohort design, [35] in order to measure the strength of the association between the two outcomes. We will test whether the (instantaneous) slope of the biomarker trajectory is associated with the study endpoint.

Both univariate and multivariate analyses will be applied. The biomarker trajectories in the linear models will be adjusted a) for age and sex, b) GRACE risk score, c) kidney function, d) body mass index, diabetes mellitus, prior CAD, prior cerebrovascular disease and prior peripheral vascular disease, and e) other variables that appear related with biomarker levels in the analysis set, to the extent that is permitted given the number of observations. The relation between biomarkers and study endpoints in the Cox model will be adjusted GRACE risk score and prognostic biomarkers, to the extent that is permitted given the number of endpoint cases. For the purpose of multivariate adjustment, we will select the specific GRACE risk model that is best in line with the purpose of our study, namely an assessment of post discharge death and MI. That particular GRACE risk model consists of age, Troponin (or CKMB) elevation at admission, history of MI, congestive heart failure and whether CABG was performed at the index hospitalization. [36]

Risk models

Based on the results of the analyses above, multi-biomarker models will be constructed to predict the risk of the study endpoints based on the temporal evolvement of the biomarkers. We realize that the number of biomarkers (and covariates) will be limited by the number of endpoint events. [37]

Early washout biomarker patterns and ancillary analyses

In an ancillary study of 68 patients (10% of the initially planned total study population of at least 700 patients), we aim to study the evolution/normalization of biomarker during the first 8 weeks after the index event. In these patients, (additional) blood samples are collected within 24, 48, 72 and 96 hours after admission, at the day of hospital discharge, and at 2, 4 and 8 weeks after discharge. Insight in these patterns will allow us to differentiate whether observed divergent biomarkers patterns prior to a repeat ACS during longer-term follow-up are (partly) influenced by biochemical consequences of the index event.

Patients will use multiple medications that might influence biomarker levels (e.g. beta-blockers, ACE-inhibitors and especially statins are known for their pleiotropic effects). However, for ethical reasons, we will not interfere with the patient's treatment. Biomarkers might also be influenced by inflammatory processes due to other illnesses. We will analyse these phenomena descriptively.

Study organization

The study is conducted under the leadership of an executive committee that has overall responsibility for protocol design, study conduct and publication. The Clinical Epidemiology unit of the Erasmus MC department of Cardiology serves as the coordinating centre for the study and oversees all activities including (out-patient) clinical follow-up, data management and statistics, as well as blood sample handling, transport and long-term storage.

Current status

BIOMArCS enrolled 844 patients between March 1st 2008 until January 26th 2015 (Table 2). Currently 1-year follow-up and event adjudication have been completed. Prespecified biomarker analyses are currently being performed and dissemination through peer-reviewed publications and conference presentations is expected from the third quarter of 2016.

DISCUSSION

Vulnerable period versus vulnerable plaque

The notion of the "vulnerable plaque" has gained currency in recent years, partly because the concept of an inflamed, rupture-prone, thin-capped fibroatheroma fits well within our current understanding of atherosclerosis biology. Still, it remains important to realise that *ex vivo* as well as *in vivo* studies using coronary intravascular ultrasound in patients with myocardial infarction have demonstrated the presence of vulnerable plaques in other than the culprit lesion or even culprit artery. [38,39] In other words, vulnerable plaques are numerous and a certain part of the plaques that we may classify as vulnerable will never disrupt. [11] Understanding of the clinical implications of the presence of vulnerable plaques becomes even more difficult given the observations that even in the case of plaque disruption and thrombus formation, this does not always imply a major symptomatic event, since many coronary thrombi remain mural and produce few if any symptoms. [40]

By selection of a clinically relevant endpoint and by analysis of biomarkers at various time points prior to the endpoint, BIOMArCS is well-suited to identify a "vulnerable period" during the follow-up of a "vulnerable patient", instead of merely detecting the presence and a certain degree of destabilisation of vulnerable plaques.

Rationale behind the time intervals for sample collection

The average time from collection of the last blood sample in asymptomatic condition until the occurrence of the coronary event will be 7 days in case of an event during the first 6 months after enrolment and 14 days during the latter half year of follow-up. Since similar studies have not been conducted before, there is a concern that altered biomarker patterns indicating an imminent event might be missed due to length of the intervals between individual samples. However, more frequent blood sampling than proposed in the current protocol would test the boundaries of an ethically acceptable burden for study patients. Furthermore it is important to realise that the longer term aim is to strive for implementation of serial multimarker testing in the routine follow-up of ambulatory patients. Recognition of distinct short-term future periods of high coronary vulnerability could in the near future serve to prevent the imminent event by intensification of treatment (by pharmacological and/or percutaneous coronary intervention) in individuals that are selected on the basis of a divergent "biomarker signature". Future long-term routine clinical follow-up of patients in an even more frequent scheme of sampling seems practically unfeasible and reliable point-of-care multimarker tests that are not semi-quantitative currently do not exist. Moreover, interventions to prevent the so-called imminent event require time as well.

Although the BIOMArCS concept is very novel, there is some, though limited, evidence that the chosen time intervals of our exploratory and clinically adaptable protocol in fact do allow observation of upregulation of pathophysiological mechanisms leading to an ACS. Rittersma et al. used pathological classification of aspired intracoronary thrombi to demonstrate that in at least 50% of patients with ST-elevation myocardial infarction, coronary thrombi were days or even weeks old. [41] This supports our hypothesis that sudden coronary occlusion is often preceded by a variable period of coronary instability and thrombus formation, initiated days or weeks before onset of symptoms. A second study evaluated formalin stored hearts and tissue blocks of coronary arteries including the thrombosed culprit plaque of young adults (≤35 years), who had died within 1 hour after onset of symptoms due to a coronary thrombotic occlusion and drew a similar conclusion. [42] A third study used platelet mRNA profiling in order to demonstrate that the expression of a certain biomarker, myeloid-related protein-14, is upregulated prior to STEMI. Because platelets are anuclear, the platelet transcriptome mirrors megakaryocyte-derived mRNAs and represents an averaged mRNA profile of variably aged platelets (platelets circulate for 7 to 10 days). [43] Finally, serial angiographic studies in the 1990s have demonstrated a sudden rapid lesion progression in weeks to months prior to myocardial infarction [10,44,45]. The possible mechanisms for such rapid plaque progression and consequent luminal obstruction include recurrent plaque rupture and healing, intraplaque neovascularization and hemorrhage with deposition of erythrocyte-derived free cholesterol. [10]

Future directions

As indicated previously, the longer term perspective of this study is to recognize distinct periods of high coronary vulnerability in individual patients days to weeks in advance, so that a tailored therapy and intensification of treatment might prevent the imminent event. Biomarker patterns and kinetics following and prior to an ACS have not been described at such short intervals during 1-year follow-up before. This study will therefore provide insight in the usefulness of combinations of certain markers for risk prediction at such short term. The descriptive data collected in this study could be used for the construction of both a short- and longer-term multimarker risk prediction model. Current risk prediction models are generally characterized by their use of baseline patient characteristics and lack of account of disease characteristics and progression over time. A multimarker approach, in which a combination of different biomarkers actually reflects atherosclerosis biology and dynamics, might therefore improve overall risk prediction. Of course, such an assertion also implies epidemiological challenges. Prediction on the basis of short term repeated measurements that reflect risks that are dynamic over time, instead of linear and continuous, requires alternative statistical approaches.

At a later stage (and dependent on the results of the above mentioned projects), the way could be paved towards intervention studies that evaluate the effectiveness and safety of a brief period of intensified medical treatment (or a percutaneous intervention) in order to prevent an otherwise imminent coronary event, as characterized by an abnormal "high-risk" biomarker pattern. Future hypotheses could focus on plaque stabilization or regression and endothelial repair in patients with "high-risk" biomarker patterns such as a brief period of intravenous administration of Apolipoprotein-A1 Milano [46], Proprotein Convertase Subtilisin/Kexin Type 9 Inhibition [47], or the use of the anti-inflammatory properties of P-selectin antagonists [48], low-dose colchicine [49], low-dose methothrexate or interleukin-1β inhibition [50]. Perhaps divergent biomarker patterns could be evaluated for selection of patients that benefit from prolonged dual antiplatelet therapy. Exogenous drugs amongst which agonists of vascular endothelial growth factor, peroxisomal proliferative activated receptor agonists and granulocyte-colony-stimulating factor, which exert their actions partly through endothelial progenitor cell-mediated re-endothelialisation may be of interest as well. [51]

Obviously, the data generated by this study could also be used for the identification of individuals with a "low risk" biomarker pattern. Tailored therapy for them might imply a reduction in pharmacological treatment regimes.

Strengths and limitations

BIOMArCS is the only currently available study in which such frequent blood sampling has been performed on a large scale in order to thoroughly investigate multiple biomarker patterns in patients with coronary artery disease. As such, BIOMArCS is conceptionally different from all other biomarker studies in patients with coronary artery disease, as it aimed to obtain blood samples as shortly as possible **prior** to a future adverse cardiac event. Although sample collection was performed prospectively, biomarker and genetic analyses will be performed retrospectively. As a dedicated biomarker study if benefits from a strict and prespecified laboratory processing protocol in which pre-analytical confounding was minimized through standardization of methods and materials for blood collection in all centers. Time from collection to standardized processing and freeze and thaw cycles for biomarker analyses are limited by protocol. Patients were interviewed at each venapuncture to inquire on their cardiac status and medication use, but also to inquire on confounders of specific biomarkers (e.g. new onset of other illnesses, infection, allergic reactions.)

It is important to emphasize that a clinical observational study as BIOMArCS does not aim to unravel whether certain biomarkers are merely markers reflecting pathways of disease, or mediators that are directly involved within distinct pathophysiological cascades in the arterial wall. Definite delineation of biochemical events responsible for observed alterations in biomarker patterns prior to the endpoint, or final conclusions on

mechanisms of disease are beyond the scope of this study design. In addition, our study was performed in patients with known coronary artery disease. It is uncertain whether its conclusions may be extrapolated to the primary prevention setting.

Collaboration

Anyone can submit a prespecified analytical plan for biomarker analyses within the BIOMArCS data set to the principle investigator/Clinical Epidemiology unit of the Erasmus MC department of Cardiology.

Biomarker analyses can only be perfomed after evaluation and written approval thereof by the BIOMArCS Executive Committee.

Contributorship statement

Rohit M. Oemrawsingh (RMO), K. Martijn Akkerhuis (KMA), Eric Boersma (EB) and Maarten L. Simoons (MLS) were responsible for the design of the BIOMArCS study. RMO drafted the manuscript. All other authors, KMA, EB, MLS, V.A. Umans, B. Kietselaer, C. Schotborgh, E. Ronner, T. Lenderink, A. Liem, D. Haitsma, P. van der Harst, F.W. Asselbergs, A. Maas, A.J. Oude Ophuis, B. Ilmer, R. Dijkgraaf, R-J de Winter, S.H.K. The, A.J. Wardeh, W. Hermans, E. Cramer, R.H. van Schaik, I.E. Hoefer, P.A. Doevendans, revised it critically for important intellectual content and approved this version to be published. All authors contributed substantially to data acquisition for the BIOMArCS study and agree to be accountable for all aspects of this work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests

BIOMArCS was designed and initiated by the principle investigators. The trial will be conducted, and its results interpreted and reported independently of the aforementioned sponsors. All authors declare that there is no conflict of interest; no financial relationships with any organisations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

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CORONARY VULNERABILITY

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6

TEMPORAL EVOLVEMENT OF HIGH-SENSITIVITY CARDIAC TROPONIN SERUM CONCENTRATIONS DURING 1 YEAR AFTER ACUTE CORONARY SYNDROME ADMISSION

Submitted

ABSTRACT

Background: Serum levels of high-sensitivity troponin (hsTn) are elevated in patients admitted for acute coronary syndrome (ACS). Detailed insights in the biological variation of hsTn and its temporal pattern following ACS are currently not available.

Purpose: To describe the temporal evolvement of hsTnI and hsTnT after admission for ACS, and to determine their variation during the clinically stable phase up to one year thereafter.

Methods: BIOMArCS is a prospective, observational study with high frequency blood sampling during one year post-ACS. 1507 blood samples from 191 patients who remained free from major adverse cardiac events (MACE) during follow-up were used to analyze the temporal evolvement during follow-up with linear mixed models. Biological variation was studied using the samples collected in the timeframe of 6-12 months after the index ACS, when patients were considered to have stable coronary artery disease.

Results: On average, both hsTnI and hsTnT were clearly elevated during ACS and remained elevated for 43 (hsTnI) and 15 days (hsTnT). The intra-individual variation in the 6-12 months window was 14.0% and 18.1% for hsTnI and hsTnT respectively, while the inter-individual variation was 94.1% and 75.9%. Using the first two samples taken from one month after the index ACS onwards, we were able to compose a patient-specific reference value for over 80% of the patients.

Conclusions: HsTnI and hsTnT remain elevated for a prolonged period after ACS. Given the low intra-individual and the large inter-individual differences, we propose further investigation of the use of patient-specific reference values over population-derived ones. Such patient-specific reference values can be adequately determined in the majority of patients using just two consecutive samples.

INTRODUCTION

High-sensitivity Troponins (hsTn) are now commonly used in clinical practice. In patients presenting with ischemic chest pain, serial cardiac hsTn measurements with at least one value above the 99th percentile of a healthy control group, in combination with a typical rise and/or fall of the serial measurements, are key elements of the diagnosis of myocardial infarction.[1,2] In addition, hsTn levels have also been demonstrated as prognostic markers in acute coronary syndrome (ACS) patients and in patients with known coronary artery disease (CAD). We demonstrated that asymptomatic post-ACS patients with mildly elevated hsTns have a doubled incidence of repeat ACS within one year.[3] Similar results were described by Ang et al in a study among 326 consecutive ACS patients and by Koenig et al among 1050 patients with previous hospitalization for ACS or coronary artery bypass grafting, associating persistently elevated hsTnT at approximately 7 weeks after index event with increased risk of cardiovascular events during a long-term follow up.[4,5]

Both in the context of diagnosing future re-ACS as well as in the context of personalizing the prognostication for future re-ACS, it is critical to be able to separate dynamic troponin changes due to myocardial necrosis, from their analytical and biological fluctuations. To date, studies on the biological variation of cardiac troponins, measured with contemporary high-sensitivity assays, are scarce and their sample sizes have usually been small.[6-10] Particularly in patients with known CAD or post-ACS patients, little to no information is available. Interpreting troponin values after ACS, is further complicated due to the prolonged period that the troponin value can remain elevated above the 99th percentile of normal.[11-13]

Against this background, we utilized the 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) with highly frequent blood sampling, investigating stabilization patterns of hsTnI and hsTnT after ACS. In particular, we aimed to determine the time until their stabilization, and, once stabilized, their intra- and inter-individual variation.

METHODS

Patients

The study design of BIOMArCS has been published previously.[14] In short, BIOMArCS is a multi-centre, prospective, observational study that was conducted in 18 participating hospitals in the Netherlands during 2008-2015. The study was designed to obtain detailed data on biomarker patterns until one-year follow-up post-ACS. Patients above 40 years presenting with ACS and at least one additional cardiovascular (CV) risk factor

were eligible for enrolment. Exclusion criteria were ischemia precipitated by a condition other than atherosclerotic CAD, a left ventricular ejection fraction <30%, or end-stage congestive heart failure (NYHA class >= 3), severe chronic kidney disease, or a coexistent condition with life expectancy <1 year. All patients were treated according to prevailing guidelines and at the discretion of the treating physician. The study protocol was approved by the Institutional Review Board of the participating hospitals, and all study subjects gave written informed consent.

Blood sampling and storage

Blood samples were collected at admission, at the day of hospital discharge and subsequently every fortnight during the first six months after discharge. If logistic circumstances hindered inclusion during hospitalization, patients could be included on the first outpatient visit within 6 weeks after discharge. In a subset of approximately 8% of patients, additional blood samples were collected within 24, 48, 72 and 96 hours after admission and at the day of hospital discharge with the specific aim to study the early evolution and normalization of the biomarkers. Follow-up was terminated permanently after coronary artery bypass grafting, hospital admission for heart failure, or a deterioration of renal function leading to a glomerular filtration rate <30ml/min/1.73 m^2.

Blood samples were handled and securely stored on-site. After preparation, aliquots were frozen at –80 degrees Celsius within two hours after withdrawal. Samples were transported under controlled conditions to the department of Clinical Chemistry at the Erasmus MC for long-term storage.

Study patients and troponin analysis

For the analysis of the BIOMArCS study, hsTnI and HsTnT serum levels were measured in the samples of 187 patients. Of these 187 patients, 45 had a new ischemic event during the follow-up. For the current analysis, we removed the patients with a new ischemic event from the analysis set and enriched the set with 49 patients who had daily sampling during the first 4 days of the index ACS submission. Hence, our analysis set consisted of 191 endpoint-free patients. They contributed a median of 8 (25^{th}-75^{th} percentile 5-10) repeated samples per patient (altogether 1507 samples) that were used for the analysis of stabilization patterns.

Based on previous studies we presumed that hsTn levels would be biochemically stable at 6 months post-ACS.[15-17] Accordingly, the analysis of biological variation was based on 446 samples that were collected 6-12 months after the index ACS, and was limited to the 98 patients who had ≥3 measurements in that time window and who did not undergo a (staged) percutaneous coronary intervention (PCI) – thus iatrogenic distortion of the troponin levels caused by PCI was excluded.[18]

Troponin values were determined in a blinded fashion and in one batch using a hsTnI assay (Abbott) and a hsTnT assay (Roche). These assays have a lower limit of detection and upper reference limit (99th percentile) of 1.2 ng/L and 10 ng/L for hsTnI, and 5 ng/L and 14 ng/L for hsTnT, respectively.

Measures of biological variation

The coefficient of variation (CV) of a series of measurements is defined as 100% times the standard deviation (sd) of the measurements divided by their mean value ($\overline{X}$):

$$CV = 100\% * sd/\overline{X}$$

According to the methods by Fraser and Harris,[19] the total variation of a series of repeated measurements in individual subjects can be split in 3 components, which represent the variation due to the imprecision of the analytical process (CV_a), the intra-individual or within-subject variation (CV_i) and the inter-individual or between-subject variation (CV_g). CV_a can be determined by repeatedly measuring the same sample using different assays. However, since this procedure is expensive, time-consuming and resource draining, laboratories generally use the CV_a that is based on a reference sample. We used the lab-specific CV_a of 5.0% for hsTnI and 3.0% for hsTnT, respectively. Besides determining the different coefficients of variability, we also calculated the *Index of Individuality (II)* and the Reference Change Value (RCV) for both biomarkers. The II is the ratio of the combined within-subject and analytical variation relative to the between-subject variation. Previously it has been suggested that in case of an II <0.6, individual subjects should have their own reference values instead of a population based reference.[20] When the II >1.4, a population-based reference is preferred. The RCV reflects the limit of (relative) change in biomarker values in individual subjects that can be explained by the combined within-subject and analytical variation. A more detailed description of the parameters of variability and the formulas used to calculate them are included in the *supplementary files*.

Data analysis

Continuous variables are presented as mean (SD) or median (25th-75th percentile), depending on their distributions. Categorical variables are summarized as numbers and percentages. We used linear regression for investigating which factors were associated with the CV_i.

Stabilization

We used linear mixed models to describe the average troponin stabilization patterns over time. In these models, time was entered as the independent variable, and the log-

transformed troponin value as the dependent variable. A total of three cubic splines were placed in order to model the non-linearity of the association between time and troponin level. We used Akaike's information criterion and Bayesian information criteria for the optimal placing of these splines. Random slopes as well as random intercepts were included in the models to allow for individual variation. Using the mixed model, we calculated the average daily values of both hsTnI and hsTnT. These values were then used to determine the average time during which troponins were raised above the reference value after ACS, and the average time until stabilization. We defined stabilization (on group level) as a difference in (model-derived) average troponin level of less than one percent between two consecutive measurements.

The analyses of the post six-month blood samples revealed a low II for both hsTnI and hsTnT. Hence, individual based reference values are preferred in our ACS patients, which ideally, are to be known as early as possible. Since the average time until stabilization appeared less than a month for both troponins, we performed a post-hoc analysis, based on the samples taken after one month, to learn if patient-specific reference values for hsTnI and hsTnT can be determined in this early time window.

We calculated the average of the first two hsTn measurements and compared this with the first consecutive measurement. If the difference was less than 5 ng/L, we considered the average of the first two measurements to be the patient-specific reference. If a patient-specific reference value was observed, we verified this value by comparing it with the last available measurement of the same patient for differences larger than 5 ng/L and by using paired t-test. The 5 ng/L threshold is equal to the median patient-specific hsTnT level times the upper limit of the RCV.

All analyses were performed using R 3.1.1.

RESULTS

Baseline characteristics

Baseline characteristics are presented in table 1. The mean age of the patients in the analysis set was 63.0 (11.1) years and 78% were men. More than half of the population had hypertension (52.1%) and a large proportion had hypercholesterolemia (47.5%) and/or a family history of CAD (53.5%). STEMI was the most common index event (46.2%), followed by NSTEMI (40.7%). No relevant differences in baseline characteristics could be identified when comparing the analysis set (n=191) and the 122 and 98 patients with more than three readily available Tn measurements after 1 and 6 months of follow-up.

Table 1. Baseline characteristics

	Analysis set (n = 191)	Post 6 months (n=98)
Age. Y (SD)	62.4 (10.6)	62.8 (9.5)
Male gender (%)	148 (77.5)	77 (78.6)
Cardiovascular risk factors (%)		
Diabetes Mellitus	33 (17.3)	17 (17.3)
Hypertension	101 (52.9)	52 (53.1)
Hypercholesterolemia	92 (46.5)	54 (58.2)
Family history of CAD	87 (53.0)	47 (59.5)
Current smoker	80 (41.9)	41 (41.8)
History of cardiovascular disease (%)		
MI	50 (26.2)	30 (30.6)
CABG	14 (7.3)	6 (6.1)
PCI	44 (23.2)	28 (28.9)
Stroke	19 (9.9)	7 (7.1)
Admission diagnosis (%)		
STEMI	93 (49.0)	47 (48.0)
NSTEMI	74 (38.7)	37 (37.8)
UAP	24 (12.6)	14 (14.3)
Revascularisation during index admission		
PCI	147 (82.1)	79 (86.8)
CABG	5 (2.6)	1 (1.1)
Physical examination		
Body mass index (SD)	27.5 (3.6)	27.5 (3.6)
Killip class 1 (%)	177 (92.7)	94 (95.9)
Heart rate (IQR)	73 (62-84)	70 (61-81)
Systolic blood pressure (IQR)	137 (117-152)	136 (119-151)
eGFR, ml/min/1,73 m^2 (SD)	98 (30)	97 (28)
Discharge medication (%)		
Aspirin	183 (96.3)	95 (96.9)
BetaBlocker	167 (87.9)	83 (84.7)
ACEi	138 (72.6)	68 (69.4)
ARB	22 (11.6)	11 (11.2)
Statin	183 (96.3)	96 (98.0)

SD: Standard deviation; IQR: Interquartile range; Y: year; CAD: coronary artery disease; eGFR: estimated glomerular filtration rate; MI: Myocardial infarction; CABG: coronary artery bypass grafting; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction; NSTEMI: non ST-elevation myocardial infarction; UAP: unstable angina pectoris

Post 6 months: Analysis set minus (1.) an elective PCI more than 150 days after the index event and (2.) patients with less than 3 samples available after 6 months.

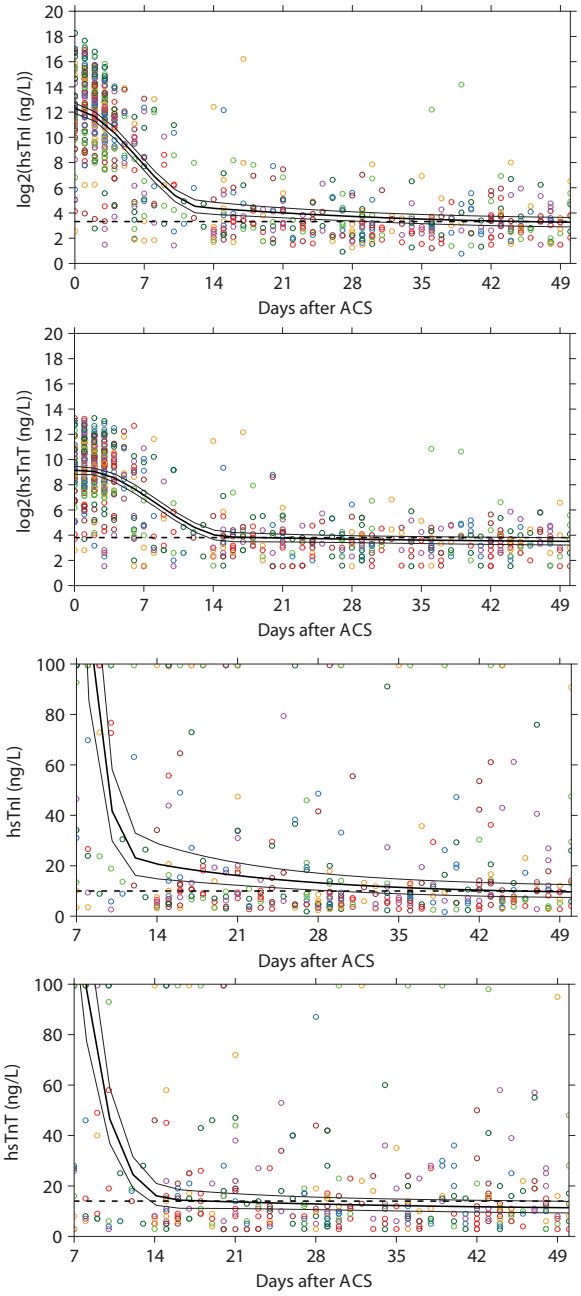

Figure 1. Average stabilization patterns of high-sensitivity troponins after ACS
The X-axes depict the number of days since the ACS. The Y-axes represent the troponin levels. The upper two plots are on the log scale with base number 2. A 1 point increase can thus be interpreted as a doubling of the value. The black lines depict the cohort average; the dashed lines the corresponding 95% confidence interval.

Temporal evolvement after the index ACS

The average concentrations of the different biomarkers from the time of the ACS until day 50, are shown in Figure 1. Both hsTnI and T were clearly elevated at the onset of ACS and gradually returned to levels beneath the reference values. The moment at which biomarker levels did stabilize was 20 days for hsTnI and 16 days for hsTnT. However, hsTnI remained above the population reference value on average almost three times longer (43 days) than hsTnT did (15 days).

Biological variation

The distributions of the hsTn measurements after 6 months are shown for each patient in figure 2. None of the samples had a hsTnI level below the detection limit; 14.0% had a hsTnT level below the detection limit (but none were below the limit of blank). In total 12.6% of the values were above the population reference value for hsTnI and 17.3% for hsTnT.

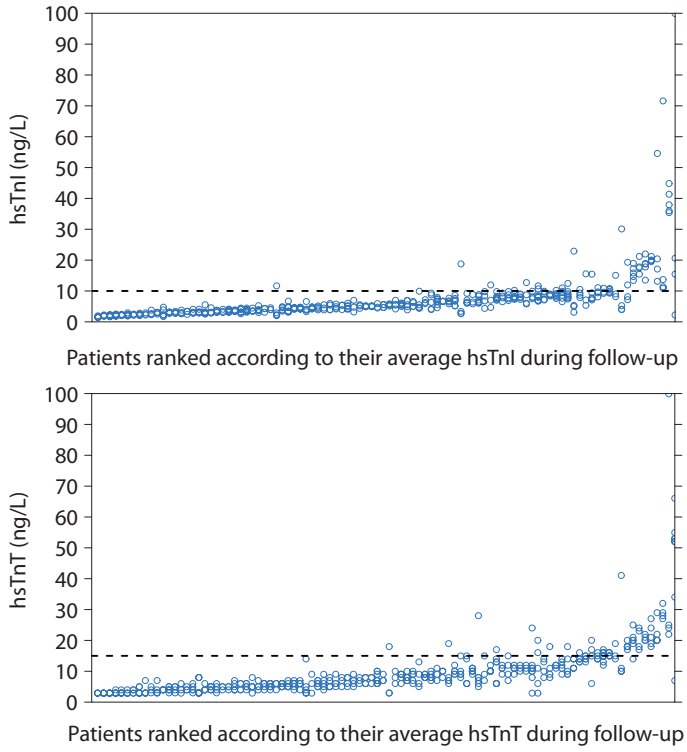

Figure 2. Distribution of the high-sensitivity troponins after six months
On the horizontal axes are the individual patients ranked based on their average troponin values. The vertical axes depict the troponin levels resulting from the repeated measurements. The dotted lines show the reference value of the troponin

The CV_i was slightly higher for hsTnT. We could not identify any baseline characteristics that were significantly associated with the observed CV_i (supplementary table 1). In contrast to the small CV_is, the CV_gs were large, reflecting relatively large differences in average troponin levels between patients. Consequently, both biomarkers had II's <0.6, The RCV limits ranged between −33.6% and 50.5% for hsTnI and −39.6% and 65.5% for hsTnT, respectively. In practice, this means that for a patient with a steady state hsTnI level of 5 ng/l, the maximum hsTnI rise based on analytical and within-subject variation is 3 ng/l. A larger increase would thus most likely be caused by pathological processes. An overview of the different parameters for the biological variation is presented in table 2.

Table 2. Overview of parameters of biological variation

							Log-normal	
	Mean patient level	CVa	CVi	CVg	II	RCV(%)	RCV low (%)	RCV up (%)
HsTropI	5.3 (3.7-8.3)	5.0	14.0	94.1	0.16	38.7	−33.6	50.5
HsTropT	7.8 (5.1-11.1)	3.0	18.1	75.9	0.24	50.1	−39.6	65.5

HsTropI: high-sensitivity troponin I; HsTropT: high-sensitivity troponin T; CVa: analytical coefficient of variation; CVg: interindividual coefficient of variation; CVi: intraindividual coefficient of variation; II: index of individuality; RCV: reference change value

Patient-specific reference

In a total of 122 patients, with 3 or more samples post 30 days, a patient-specific reference values could be determined in 85.2% (hsTnI) and 83.6% (hsTnT) using the first two post-30 day measurements. The median (IQR) reference value was 7.1 ng/L (4.4-10.6) for hsTnI and 8.5 ng/L (6.5-12.9) for hsTnT. In addition, we compared the observed patient-specific references values with the last available measurement from the same patient. The difference between the patient-specific reference value and their last available measurement was less than 5 ng/L in more than 81.7% (hsTnI) and 77.5% (hsTnT) of the patients. A paired t-test confirmed that there were no significant differences between the two values for both hsTnI (mean 10.0 vs 10.4 ng/L, p = 0.80) and hsTnT (mean 10.5 vs 10.4 ng/L, p = 0.91).

DISCUSSION

In BIOMArCS we observed that after the hsTn peak and plateau of the index ACS, hsTnT reached values below the clinically used reference value faster than hsTnI. The individual variation of hsTnI and hsTnT was low, while the differences between patients were large. This combination of characteristics led to a low II (<0.6) for both troponins, which stresses the need for a patient-specific instead of a population-based reference hsTn value[20] in patients with known stable CAD after having previously endured an ACS. We

demonstrated that in the majority of the patients two samples taken after at least one month sufficed to find such a reference value.

The parameters of variation are comparable to earlier reports in healthy populations. A study by Wu et al. of 17 healthy subjects also found a long-term individual variation of 14% for hsTnI.[9] The CV_g in their report was low (63%) in comparison to our study which indicates that there is a larger variation in troponin levels in stable ACS patients than in healthy individuals. The larger between-subject variation in a diseased population compared to a healthy one, is also confirmed by a study of Meijers et al. comparing biological variation in 83 patients with heart failure to 28 healthy subjects.[21] They reported a CV_g for hsTnT of 96.6% and 51.2% respectively. The CV_is however, were similar in both populations and comparable to our cohort.

Our data, as well as earlier research, all showed a low II for both hsTnI and hsTnI which means that a patient specific reference value is preferred over a population based reference value. We are the first to demonstrate that such a value could be retrieved in the majority of patients based on a limited number of consecutive measurements. These reference values showed good agreement with samples taken later during the year of follow-up. In a real life setting, the method could even be further optimized. If a treating physician identifies a patient with a prolonged stabilization the following measurement could be postponed. Also outliers could be neglected. Although it would take some extra visits of the patient for blood sampling to the clinic, determining the patient specific reference value could provide clinical benefits by leading to a more precise diagnosis of ACS or better rule-out. Furthermore, using our method, we can identify patients with a patient specific reference value above the population based reference value. In BIOMArCS we observed that in clinically asymptomatic patients that had endured an ACS more than 6 months ago, 12.6% of the hsTnI and 17.3% of the hsTnT values were above the population reference value, i.e. 99th percentile of a healthy reference population. This is exactly in line with our previous observation in the ATHEROREMO study in which hsTnT was above the 14 ng/L cut-off in 19.5% of 212 stable CAD patients.[22]

A recent study conducted at the emergency department of the Karolinska University Hospital also identified that approximaly one fifth, i.e. 4052 out of 21189 chest pain patients without ACS, present with an initial hsTnT concentration above 14 ng/L, once again confirming that an initial hsTnT elevation outside the setting of an ACS is a common finding in the emergency department.[23]

Similarly, the fact that a large percentage of patients with known stable CAD has chronic Tn elevation above the 99th percentile has to be accounted for when evaluating these patients in the emergency room. A patient specific reference value could aid the diagnostic process in such cases. Finally, the patient specific reference is also useful in determining the prognosis of the patient since there is a strong and graded association between hsTn and adverse outcome.[3,15,23-26]

At this point, there is no clear definition of the transition point at which post-ACS patients are considered to be stable CAD patients. From a biomarker perspective, our data show that all ACS-patients reach a point after which the Troponins remain within a constant, patient-specific range.

Due to the highly-frequent blood sampling, the BIOMArCS provides a unique platform to study longitudinal biomarker patterns in a real-world post-ACS population. However, a limitation is that the study protocol did not specify the time of sampling during the day and that we have no information of the patients activity prior to sampling. HsTns are known to be influenced by (heavy) physical activity[27] and to have a circadian rhythm.[28] We have investigated the variation of the time of sampling and found that all measurements were taken between 8 o'clock in the morning and 4 o'clock in the afternoon. Moreover, we found that, although not specified in the protocol, the vast majority of the patients always came in at the same hour for their blood sampling. Hence, the within-patient variation in biomarker levels found in this study cannot be explained by changes in sampling time. A second limitation of our study protocol is that we excluded all patients with recurrent events for determining the parameters of variability. Although correct, as we do not want to take into account possible distortion from an imminent ischemic event, this could potentially compromise generalizability of our parameters. However, in a sensitivity analysis also comprising the patients with ischemic events, the parameters only changed marginally (data not shown). A final limitation is that using our data, we cannot confirm that using a patient-specific reference value enhances the diagnostics for future ACS. This should be the focus of future research.

Conclusion

In conclusion, hsTnT levels stabilize on average after 16 days and TnI levels after 20 days after ACS, although hsTnI levels remain above the 99th percentile reference value longer (43 days) than hsTnT levels (15 days). Once the troponins have stabilized, within-patient variation is small, and comparable to healthy populations. Between-patient variation, however, is much higher in post-ACS patients than in population controls. Finally, with two samples taken at least one month after ACS, a patient-specific reference value can be determined in the vast majority of the patients. This reference value could aid in future diagnostics and could help provide an indication of the prognosis of the patient.

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SUPPLEMENTARY FILES

Methods

The CV_i was defined as the median value of the CVs of the repeated measurements in individual subjects ($CV_{subject}$), adjusted for the analytical variation:

$$CV_i = \sqrt{\text{median}(CV_{subject}^2) - CV_a^2}$$

Finally, CV_g was determined as 100% times the standard deviation ($sd_{\bar{x}_{subject}}$) of the mean values of the repeated measurements in individual subjects ($\bar{X}_{subject}$) by the (unweighted) mean of these means ($\bar{X}_{group}$):

$$CV_g = 100\% * sd_{\bar{x}_{subject}} / \bar{X}_{group}$$

The *Index of Individuality (II)* is the ratio of the combined within-subject and analytical variation relative to the between-subject variation:

$$II = \sqrt{CV_i^2 + CV_a^2/CV_g}$$

When the II <0.6, it is agreed that subjects should have their own reference values, based on previous samples.[17] When the II >1.4, a population-based reference is preferred.

The *Reference Change Value (RCV)* reflects the limit of (relative) change in biomarker values in individual subjects that can be explained by the combined within-subject and analytical variation. For biomarkers with a normal distribution, the RCV can be calculated as follows:

$$RCV = Z_{\alpha/2} * \sqrt{2(CV_i^2 + CV_a^2)}$$

where $Z_{\alpha/2}$ represents the critical value of the normal distribution for 100% * $(1-\alpha)/2$ confidence. For biomarkers with a skewed distribution a log-normal approach has been described,[18] and the RCV limits can be determined as follows:

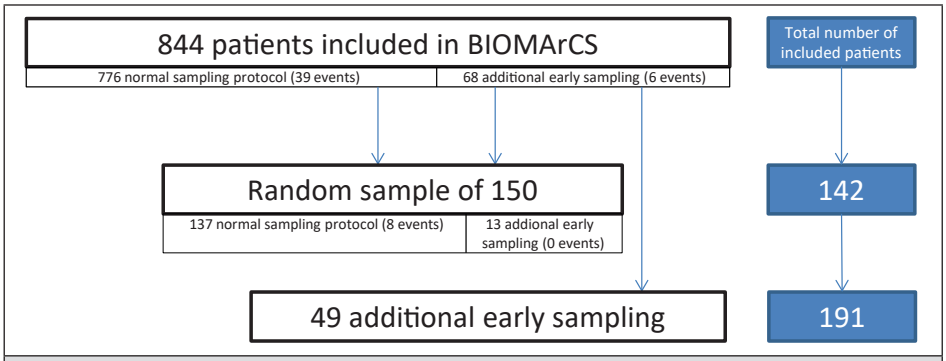
$$RCV_{downward} = e^{-Z_{\alpha/2}*\sqrt{2\ln(CV_w^2+CV_a^2+1)}} - 1$$

$$RCV_{upward} = e^{Z_{\alpha/2}*\sqrt{2\ln(CV_w^2+CV_a^2+1)}} - 1$$

We used $\alpha = 0.05$ (for 95% confidence), thus $Z_{0.025} = 1.96$.

Supplementary table 1.

	hsTnI		hsTnT	
	estimate (95%CI)	P-value	estimate (95%CI)	P-value
Male gender	0.002 (−0.686, 0.691)	0.994	−0.064 (−0.669, 0.541)	0.834
Age	−0.01 (−0.04, 0.02)	0.503	−0.022 (−0.048, 0.003)	0.088
Current Smoking	0.072 (−0.501, 0.645)	0.804	−0.15 (−0.652, 0.352)	0.555
Diabetes	−0.53 (−1.269, 0.209)	0.158	−0.213 (−0.867, 0.441)	0.519
Hypertension	−0.01 (−0.576, 0.556)	0.972	0.192 (−0.303, 0.688)	0.443
Hypercholesterolemia	0.094 (−0.474, 0.662)	0.744	−0.17 (−0.668, 0.328)	0.5
Family history of CAD	−0.061 (−0.633, 0.511)	0.832	0.193 (−0.379, 0.765)	0.503
BMI	−0.029 (−0.108, 0.05)	0.467	−0.032 (−0.101, 0.038)	0.368
Heart Rate	0.004 (−0.013, 0.021)	0.637	−0.005 (−0.02, 0.009)	0.485
Systolic blood pressure	−0.001 (−0.012, 0.009)	0.842	−0.002 (−0.011, 0.007)	0.702
Killip-class	0.782 (−0.859, −2.544)	0.947	0.52 (−0.92, −2.254)	0.717
Aspirin	0.516 (−1.122, 2.153)	0.533	0.556 (−0.881, 1.992)	0.445
BetaBlocker	−0.726 (−1.497, 0.045)	0.065	0.088 (−0.601, 0.777)	0.8
ACE inhibitor	0.059 (−0.555, 0.672)	0.85	−0.04 (−0.579, 0.498)	0.882
ARB	0.294 (−0.599, 1.188)	0.515	0.088 (−0.698, 0.874)	0.824
Statin	−1.864 (−3.827, 0.099)	0.062	−0.326 (−2.081, 1.428)	0.713



Supplementary figure 1.

CORONARY VULNERABILITY

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7

HIGH-FREQUENCY BIOMARKER MEASUREMENTS OF TROPONIN, NT-PROBNP AND C-REACTIVE PROTEIN FOR PREDICTION OF NEW CORONARY EVENTS AFTER ACUTE CORONARY SYNDROME: THE BIOMARCS STUDY

Circulation 2019 Jan 2;139(1):134-136

The *BIOMarker study to identify the Acute risk of a Coronary Syndrome* (BIOMArCS) was designed to study the relation between temporal changes in cardiovascular (CV) biomarkers and ischemic CV events in patients discharged after acute coronary syndrome (ACS) admission.[1] 844 ACS patients were enrolled in 18 hospitals in The Netherlands. Venipuncture was scheduled at 19 regular intervals during a year. Forty-five patients (cases) reached the study endpoint, defined as the first event of the composite of cardiac death (N=8), myocardial infarction (N=29), or unstable angina requiring urgent coronary revascularization (N=8) within one year. BIOMArCS was approved by the institutional review committees of the participating hospitals. All patients gave informed consent.

We used a case-cohort approach for biomarker determination and analysis.[2] The case-cohort study comprises a random subcohort from the full cohort, together with all cases. The main advantage of the case-cohort design over a cohort study is that full covariate data (in our situation: biomarker data) are only needed on the cases and subcohort individuals, not all the original cohort.[3] Thus, the advantages of a cohort study are combined with the efficiency of a nested case-control study.[3] We randomly selected a subcohort of 150 (18%) individuals, including 8 cases. Our case-cohort therefore consisted of (all) 45 cases and 142 noncases.

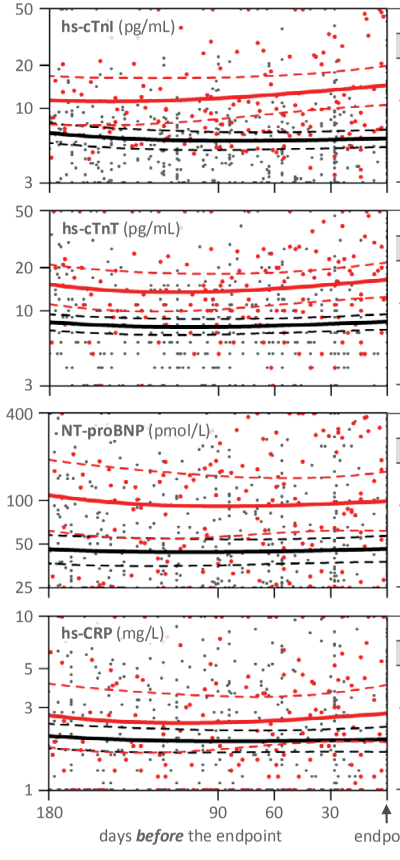
Four established CV biomarkers were then measured (in 1478 blood samples), reflecting different components of CV pathophysiology: Troponin, which was assessed with high-sensitivity cardiac Troponin I and T assays (hs-cTnI, Abbott; hs-cTnT, Roche), N-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP, validated in-house sandwich ELISA), and high-sensitivity C-Reactive Protein (hs-CRP, Beckman Coulter).[1] Biomarker measurements were performed in a single batch; personnel were blinded to any patient data.

Patient-specific longitudinal biomarker trajectories were analyzed by linear mixed effect (LME) models, with adjustment for GRACE risk score (including age), sex, clinical risk factors, recorded at inclusion, and creatinine value, recorded at each sampling moment. The relationships between biomarker levels (based on the LME models) and the endpoint were analyzed by Cox proportional hazard models. Unadjusted hazard ratio (HR) estimates for each biomarker were obtained, as well as estimates adjusted for GRACE risk score and multiple biomarkers. We applied Bayesian semi-parametric joint modeling, enabling simultaneous estimation of the LME- and Cox model parameters.[4]

Median age was 62.5 years, 77.9% were male, and 51.7% presented with ST-elevation. Measured biomarkers were elevated at the index ACS, subsequently decreased, and stabilized within 30 days. Canadian Cardiac Society angina class was ≤1 at 95.5% of the post 30-day visits, reflecting clinical stability. Renal function was preserved and stable: median (IQR) eGFR was 90 (73-114) ml/min/1.73m^2 at the final visit. Antiplatelets and statins were used at 98.7% and 95.9% of the visits.

Despite the absence of angina symptoms in the post 30-day period, cases had sustained and significant higher hs-cTnI than noncases (Figure). The mean values of

A. Temporal evolvement of cardiovascular biomarkers in cases (red) and noncases



Mean 13.0 vs. 6.7 pg/mL; P-value <0.001

Model	HR (95%CI)	P-value
Unadjusted	1.87 (1.27-2.72)	0.003
Adjusted for		
GRACE risk	1.69 (1.11-2.45)	0.015
NT-proBNP	1.47 (0.89-2.26)	0.106
hs-CRP	1.83 (1.24-2.60)	0.004

Mean 15.7 vs. 8.6 pg/mL; P-value <0.001

Model	HR (95%CI)	P-value
Unadjusted	1.87 (1.27-2.75)	0.001
Adjusted for		
GRACE risk	1.68 (1.02-2.65)	0.043
NT-proBNP	1.60 (0.93-2.55)	0.084
hs-CRP	1.94 (1.26-2.83)	0.002

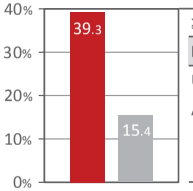
Mean 99.8 vs. 47.1 pmol/L; P-value 0.001

Model	HR (95%CI)	P-value
Unadjusted	2.38 (1.41-4.33)	<0.001
Adjusted for		
GRACE risk	2.10 (1.12-4.07)	0.021
hs-cTnI	1.94 (1.02-3.74)	0.048
hs-CRP	2.33 (1.38-4.11)	0.002

Mean 2.7 vs. 2.1 mg/L; P-value 0.138

Model	HR (95%CI)	P-value
Unadjusted	1.46 (0.82-2.62)	0.209
Adjusted for		
GRACE risk	1.18 (0.64-2.16)	0.597
hs-cTnI	1.40 (0.78-2.42)	0.250
NT-proBNP	1.37 (0.73-2.35)	0.308

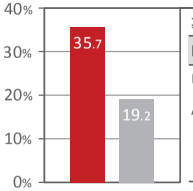
B. Percentage of cases (red) and noncases with ≥1 isolated biomarker peak value exceeding the Reference Change Value



≥1 isolated hs-cTnI value >10 pg/mL

Model	HR (95%CI)	P-value
Unadjusted	4.69 (1.90-11.6)	0.001
Adjusted for		
GRACE risk	3.81 (1.40-10.4)	0.009
hs-cTnI *	2.90 (1.04-7.27)	0.045

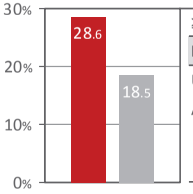
* patient-specific longitudinal hs-cTnI level



≥1 isolated hs-cTnT value >10 pg/mL

Model	HR (95%CI)	P-value
Unadjusted	2.96 (1.19-7.34)	0.020
Adjusted for		
GRACE risk	2.33 (0.84-6.48)	0.104
hs-cTnT *	1.87 (0.76-4.47)	0.171

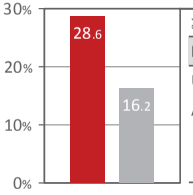
* patient-specific longitudinal hs-cTnT level



≥1 isolated NT-proBNP value >100 pmol/L

Model	HR (95%CI)	P-value
Unadjusted	3.16 (1.23-8.10)	0.017
Adjusted for		
GRACE risk	2.79 (1.07-7.25)	0.035
NT-proBNP *	1.58 (0.60-3.87)	0.332

* patient-specific longitudinal NT-proBNP level



≥1 isolated hs-CRP value >10 mg/L

Model	HR (95%CI)	P-value
Unadjusted	3.20 (1.23-8.37)	0.018
Adjusted for		
GRACE risk	3.08 (1.16-8.19)	0.025
hs-CRP *	2.92 (1.13-7.00)	0.023

* patient-specific longitudinal hs-CRP level

Figure. Temporal evolvement of established cardiovascular biomarkers and biomarker peak values in cases who reached the study endpoint (red dots and lines) and noncases

Data represent all measurements that were obtained in the post 30-day period after the index ACS. A total of 30 patients reached the study endpoint in this period (15 endpoint cases occurred in the first 30 days).

Panel A shows the temporal evolvement of biomarkers until the study endpoint (t=0 in cases), or until the last blood sample moment (t=0 in noncases). Dots represent measurements in individual cases (red) and noncases. Solid, bold lines represent group mean values, and dashed lines the corresponding 95% CIs, based on linear mixed effect models.

Hazard ratios for the study endpoint are are calculated for a 1 standard deviation increase of the biomarker (on the log scale) at any time point, and are based on joint models for longitudinal and survival data. We present unadjusted HRs, and HRs adjusted for a) GRACE risk and b) multiple biomarkers.

Panel B shows the percentage of cases (red) and noncases with ≥1 isolated biomarker peak value exceeding the Reference Change Value.

Hazard ratios for the study endpoint are calculated for a biomarker peak value above the Reference Change Value,[5] which was 10 pg/mL for hs-cTnI, 10 pg/mL for hs-cTnT, 100 pmol/L for NT-proBNP and 10 mg/L for hs-CRP. Hazard ratios are based on joint models for longitudinal and survival data, with 'peak' modelled as a time dependent covariate. We present unadjusted HRs, and HRs adjusted for a) GRACE risk and b) the patient-specific longitudinal level of the corresponding biomarker.

ACS: acute coronary syndrome; CI: confidence interval; hs-CRP: high-sensitivity C-Reactive Protein; hs-cTnI, high-sensitivity cardiac Troponin I; hs-cTnT, high-sensitivity cardiac Troponin T; GRACE: GRACE discharge risk score for ACS patients; HR: hazard ratio; NT-proBNP: N-terminal pro-brain natriuretic peptide

the patient-specific means were 13.0 and 6.7 pg/mL (P-value <0.001). Cases also had higher hs-cTnT (15.7 versus 8.6 pg/mL, P-value <0.001), and NT-proBNP (99.8 versus 47.1 pmol/L, P-value 0.001), but not hs-CRP (2.7 versus 2.1 mg/L, p-value 0.138). Hazard ratios for the endpoint per standard deviation increase were 1.87 (1.27-2.72) for hs-cTnI, 1.87 (1.27-2.75) for hs-cTnT, 2.38 (1.41-4.33) for NT-proBNP, and 1.46 (0.82-2.62) for hs-CRP. The significant associations remained after adjustment for GRACE risk score. Cardiac Troponins and NT-proBNP were correlated (Spearman r 0.54 and 0.46 for hs-cTnI and hs-cTnT), resulting in attenuated associations with the endpoint in multimarker models (Figure).

During the asymptomatic post 30-day period biomarkers tended to remain stable in the individual patient. We did not observe a (steady or more sudden) rise in the studied biomarkers prior to the endpoint. Nevertheless, 20.4% of patients had isolated peak values of hs-cTnI above the Reference Change Value (RCV)[5] of 10 pg/mL. In a post-hoc analysis, there were no temporal associations between these peaks and the endpoint. Still, the HR for the endpoint for an incident hs-cTnI peak >RCV was 2.90 (95% CI 1.04-7.27, P-value 0.045), adjusted for the patient-specific longitudinal stable hs-cTnI level (Figure). Incident hs-CRP peaks >RCV (10 mg/L) also contained independent predictive information, but hs-cTnT (10 pg/mL) and NT-proBNP (100 pmol/L) peaks did not.

Two limitations of our work need particular attention. First, differences in biomarker levels between cases and noncases might be explained by unmeasured factors, including the severity of coronary disease and left ventricular remodeling – cardiac imaging was lacking. Second, despite the large number of measurements, the small number of events precluded full multivariable adjustment for the relation between biomarkers and the study endpoint.

BIOMArCS demonstrated that longitudinal hs-cTn and NT-proBNP elevations, and incident hs-cTnI and hs-CRP peaks were associated with coronary events in clinically stable post-ACS patients. Since the studied biomarkers did not rise prior to the event, longitudinal monitoring with these markers, within this particular sampling protocol, may not identify a high-risk timeframe in individuals.

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Conflict of Interest:

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Study registration

The Netherlands Trial Register NTR1698

Key words

Acute Coronary Syndrome, Biomarkers, Risk Prediction

Article information

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure (contact the corresponding author).

CORONARY VULNERABILITY

VULNERABLE PLAQUE

CORONARY VULNERABILITY

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8

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 2014;10(8):953-960

ABSTRACT

Aims: The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study aims to investigate the relations of genetic profile and novel circulating biomarkers with coronary plaque phenotype and vulnerability as determined by intravascular ultrasound (IVUS).

Methods and results: ATHEROREMO-IVUS is a prospective, observational cohort study of 846 patients with stable angina pectoris or acute coronary syndrome (ACS) who are referred for coronary angiography. Prior to the catheterization procedure, blood samples are drawn for biomarker measurements and genetic analyses. During the catheterization procedure, IVUS is performed in a non-culprit coronary artery. The primary endpoint is the presence of vulnerable plaque as determined by IVUS virtual histology. Secondary endpoints include the incidence of major adverse cardiac events during long-term follow-up.

Conclusions: Results from ATHEROREMO-IVUS are expected to improve our knowledge on the role of genetic profile and circulating biomarkers in relation to the development of atherosclerosis and vulnerable plaques. Assessment and early validation of the prognostic value of novel biomarkers and intracoronary imaging techniques will be performed. (Clinicaltrials.gov number: NCT01789411)

INTRODUCTION

Coronary artery disease is projected to become the largest single cause of disease-burden worldwide.[1] The traditional view that atherosclerosis is simply a lipid storage disease has been evolved, considering the growing body of evidence that genetic profile, inflammation and blood coagulation play a pivotal role in all stages of atherosclerotic disease, from endothelial dysfunction to late-stage plaque rupture.[2-5] Genetic markers and circulating biomarkers of inflammation, lipids and coagulation may potentially improve risk stratification in patients with atherosclerotic cardiovascular disease, since they provide information on the biological processes in individuals.[6-7] Furthermore, these markers may also have a role in the development of new therapeutical targets. Genome wide scanning of single nucleotide polymorphisms (SNPs) and plasma lipidomics are two potential methods to identify novel genetic and lipid-related markers of coronary artery disease. *In-vivo* intracoronary imaging may further improve coronary risk stratification. Intravascular ultrasound (IVUS) backscattering analysis allows for *in-vivo* differentiation of various plaque phenotypes and may therefore be well suited for detection of plaques that are at high risk to rupture.[8-9]

The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) is designed as an exploratory (non-pivotal) clinical study to investigate the associations between genetic profile, circulating biomarkers and coronary atherosclerosis phenotype and vulnerability as determined by IVUS virtual histology Additionally, novel intracoronary imaging techniques, including near-infrared spectroscopy (NIRS), will be explored to identify lipid core plaques in the coronary arterial wall.[10] Finally, the prognostic implications of (the combination) of established and novel biomarkers and plaque phenotypes will be studied.

METHODS

Target population

The ATHEROREMO-IVUS target population consists of patients with stable angina pectoris or acute coronary syndrome (ACS) who are referred for coronary angiography. The in- and exclusion criteria are presented in table 1. Stable angina pectoris was defined as having at least two of the following three criteria: 1. substernal chest discomfort of characteristic quality and duration; 2. provoked by exertion or emotional stress; 3. relieved by rest and/or glyceryl trinitrate.[11] ACS include ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) or unstable angina pectoris. STEMI was defined by ischaemic symptoms, persistent (>20 min) ST-segment

Table 1. Inclusion and exclusion criteria

Inclusion criteria:
1. Aged 21 years or older. 2. Presenting with stable angina pectoris (CCS angina class 1, 2, 3 or 4), unstable angina pectoris (Braunwald class 1-3, B-C), documented silent ischemia or acute myocardial infarction (STEMI and NSTEMI). 3. Eligible for coronary revascularization in the native coronary artery/arteries. 4. Willing and able to comply with the specified follow-up evaluation. 5. Willing to sign informed consent. 6. Presence of a flow-limiting stenosis (diameter stenosis ≥50% by QCA or visual estimate) that is held responsible for angina pectoris or acute coronary syndrome 7. The study vessel has not undergone percutaneous coronary intervention in the last 8 months.
Exclusion criteria:
1. Angina caused by a non-cardiac illness (Braunwald class IA, IIA, IIIA). 2. Pregnant women or women of childbearing potential who do not use adequate contraception. 3. Known allergies to aspirin, clopidogrel, ticlopdine, heparin, stainless steel, copper or a sensitivity to contrast media which cannot be adequately pre-medicated. 4. Previous participation in this study or participation in another study with any investigational drug or device within the past 30 days (study participation ends after completion of the final follow-up). 5. Life expectancy of less than one year or factors making clinical and/or angiographic follow-up difficult. 6. Planned or being status post coronary bypass surgery. 7. Planned major non-cardiac surgery. 8. Impaired renal function (creatinine>2 mg/dl or ≥150 µmol/l). 9. History of bleeding diathesis or coagulopathy. 10. History of disabling stroke within the past year.
Exclusion criteria for intravascular ultrasound and near-infrared spectroscopy:
11. Three-vessel coronary artery disease or left main disease with ≥50% stenosis. 12. Minimal lumen diameter <2mm in the segments to be analyzed within the study vessel. 13. Diameter stenosis >70% or total occlusion of the study vessel. 14. In case the study-vessel has been stented previously (>8 months ago), more than 1/3 proximal of the study vessel (at least 40mm in length) should be available for examination (i.e. outside the length of the stent plus 5mm proximal to the stent). 15. Poor left ventricular function as assessed by echocardiography or by angiography. 16. Moderate or severe tortuosity of the study segment (i.e. 2 bends >75° or one bend > 90°). 17. Known tendency for coronary vasospasm.

CCS: Canadian Cardiovascular Society; NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; QCA: quantitative coronary angiography.

elevation in two contiguous electrocardiogram (ECG) leads and a raise in cardiac enzymes.[12] Patients with acute chest pain and a typical raise and fall in cardiac enzymes but without persistent ST-segment elevation were classified as NSTEMI. Unstable angina was defined by acute or worsened chest pain without persistent ST-segment elevation and without elevated cardiac enzymes.[13]

Study sample

The ATHEROREMO-IVUS study cohort mainly consists of patients who were included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands, which is an academic tertiary referral hospital serving a population of approximately 1.9 million.

This cohort was enriched with eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib versus placebo (inclusion period 2005-2006).[14]

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.

Blood sampling

Blood samples were collected to enable genome wide scans, lipid mass spectrometry and the analysis of circulating biomarkers. Blood samples were drawn from the arterial sheath prior to the coronary angiography or percutaneous coronary intervention (PCI) procedure. Blood samples were transported to the local clinical chemistry laboratory for further processing (i.e. centrifugation followed by serum, citrate- and EDTA-plasma aspiration and buffy coat separation from the EDTA tube) and storage at a temperature of −80°C within two hours.

Genome wide scans

Genome wide scans are preformed to identify a set of genetic variants that correlate with the extent and phenotype of coronary atherosclerosis. The Affymetrix GeneChip Human Mapping 6.0 Array is used for the genome wide scans of 906,600 SNPs. Quality control was performed, including correction for population structure, removal of related samples or samples with mismatched gender.

Lipid extraction and mass spectrometry

An aliquot of plasma or serum is subjected to lipid extraction. Known amounts of internal standards are added to the samples before extraction and the final lipid extracts are dried under nitrogen. The extracts are reconstituted as described elsewhere.[15] Sphingolipids are analyzed on a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Analytical Technologies) equipped with an ultra-high pressure liquid chromatography (UHPLC) system; CTC PAL autosampler (Leap Technologies) and Rheos Allegro UHPLC (Flux Instruments) using multiple reaction monitoring.[16] Shotgun lipidomics is performed by multiple precursor ion and neutral loss scanning on a QTRAP® 5500 mass spectrometer (Applied Biosystems/MDS Analytical Technologies) equipped with a robotic nanoflow ion source NanoMate HD (Advion).[17] Mass spectrometry data files are processed using MultiQuant™ 1.1.0.26 or Lipid Profiler™ (Applied Biosystems/MDS Analytical Technologies).[18] Identified lipids are quantified by normalizing against their respective internal standard and tissue wet weight for aorta and volume for plasma. Quality control (QC) samples are utilized to monitor the overall quality of the lipid ex-

traction and mass spectrometry analyses.[19] The QC samples are mainly used to remove technical outliers and lipid species that are detected below the lipid class based lower limit of quantification (LLOQ).

Intravascular imaging

Following the standard coronary angiography, eligibility for intracoronary imaging was assessed. IVUS data was acquired in a non-culprit coronary vessel. 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.

A number of selected patients in the Erasmus MC also participated in the ATHEROREMO-NIRS substudy (details are described in the supplement). In these patients, NIRS was performed in the same segment of the non-culprit vessel.

IVUS virtual histology

The IVUS gray-scale and IVUS radiofrequency backscatter analyses, also known as IVUS virtual histology, were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. The baseline IVUS images were analyzed offline in an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands). The core laboratory personnel were blinded for baseline patient characteristics as well as for biomarker, genetic and clinical outcomes data. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Extent and

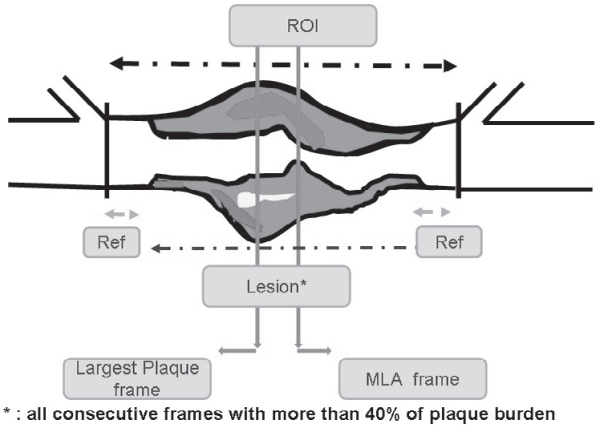

Figure 1. Methodology for detection of lesion and reference segments
MLA: minimal luminal area; REF: reference segments; ROI: region of interest.

phenotype of the atherosclerotic plaque were assessed. Plaque burden was defined as the plaque and media cross-sectional area divided by the 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 (Figure 1). Using IVUS radiofrequency analyses, the composition of the atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.[8] In consensus sessions with three investigators who were blinded to the patient characteristics and outcomes, the lesions were further classified into different lesion types (Table 2).[9] A thin-cap fibroatheroma (TCFA) lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen. Remodeling of a lesion was assessed by means of the remodelling index, expressed as the external elastic membrane cross-sectional area at the site of minimal luminal area divided by the reference external elastic membrane cross-sectional area. The reference site was selected <10 mm proximal to the lesion, with no major side branches between the site of the minimal luminal area and the reference.

Table 2. Classification of lesions on intravascular ultrasound

Lesion Type	Definition
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	Lesion 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. Thin-cap fibroatheroma	Presence of >10% confluent necrotic core in direct contact with the lumen
8. Calcified thin-cap fibroatheroma	Thin-cap fibroatheroma containing >10% of confluent dense-calcium

Follow-up

Clinical follow-up started at inclusion and will last for at least 1 year. Post-discharge survival status will be obtained from municipal civil registries. Post-discharge rehospitalizations will be prospectively assessed during follow-up. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) will be sent to all living patients. Treating physicians and institutions will be contacted for additional information whenever necessary. If possible and clinically relevant, culprit and non-culprit

lesion related events will be distinguished. The occurrence of MACE will be adjudicated by an independent clinical events committee on the basis of original source data and without knowledge of other patient, biomarker, genetic or intracoronary imaging characteristics.

Study endpoints

The primary objective of ATHEROREMO-IVUS is to correlate genetic markers and circulating (lipid) biomarkers with coronary plaque phenotype as determined by IVUS virtual histology. Therefore, the primary endpoint is defined as the presence of TCFA lesions on the imaged non-culprit coronary segment.

The secondary objective is to assess the prognostic value of established biomarkers, novel genetic and lipid biomarkers and plaque phenotypes as determined by IVUS virtual histology. Therefore, the secondary endpoint is defined as the 1-year incidence of MACE, which includes all-cause mortality, ACS or unplanned coronary revascularization. All-cause mortality is defined as death due to any cause.[20] ACS was defined as the clinical diagnosis of STEMI, NSTEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology.[21] Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG) due to progressive angina or ACS.

Sample size

ATHEROREMO-IVUS is designed to explore multiple relations. Its sample size is fixed at 800 patients, which is sufficient to reveal relations between 'abnormal' biomarkers (of any kind) and the presence of TCFA lesions with reasonable statistical certainty. We acknowledge that confirmatory studies might be required to more firmly establish relations that we will discover.

Based on prior studies, we expected that 30% of the patients will have a TCFA lesion in at least one coronary artery (i.e. TCFA positive), while 70% of the patients will not have any TCFA lesion (i.e. true TCFA negative) (Figure 2).[9] Since TCFA lesions are more or less randomly distributed across the coronary system, we expected that 10% of the patients will have a TCFA lesion in the imaged coronary artery (i.e. classified as TCFA positive), while 90% of the patient will not have a TCFA lesion in the imaged coronary artery (i.e. classified as TCFA negative). In patients who are classified as TCFA negative, 77.8% is expected to be true TCFA negative.

A biomarker level in the upper quintile of its sample distribution is considered as 'abnormal'. We expect to observe abnormal biomarker levels in 5-15% of the true TCFA negative patients and in 10-40% of the TCFA positive patients. Hence, the proportion of abnormal biomarker levels in the patients who are classified as TCFA negative are expected to range from 6.1-20.6% (Table 3). Table 4 presents the statistical power to

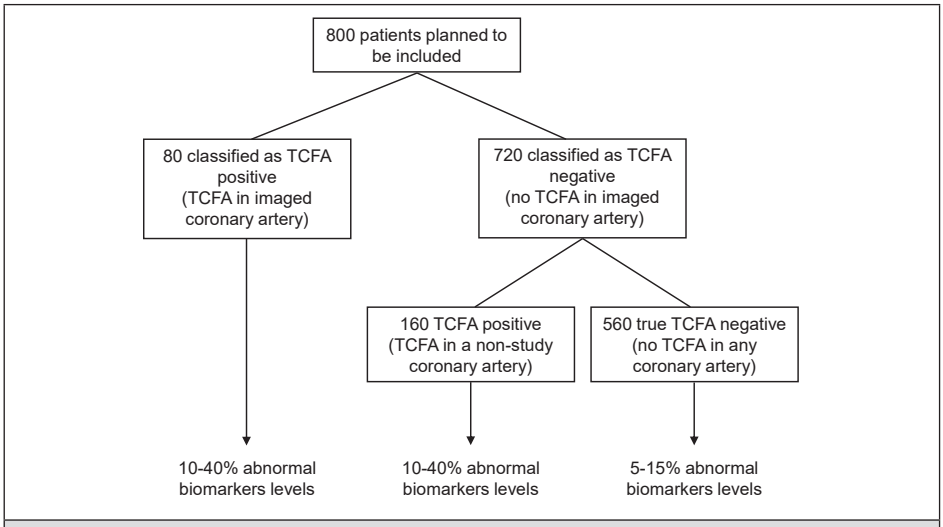

Figure 2. Expected prevalence of thin-cap fibroatheroma lesions
TCFA: thin-cap fibroatheroma.

detect differences in the frequency of abnormal biomarker levels between patients who are classified as TCFA negative versus those who are classified as TCFA positive (α-error 5%, two-sided test). The power is adequate (≥80%) for the most realistic scenarios.

Power calculations were not performed for the secondary endpoint. Based on the results of other studies and previous registries in our hospital, we expect that MACE will occur in 5-10% of the patients within the first year of follow-up.[22-25]

Table 3. Expected percentage of patients with abnormal biomarker levels

		Frequency of abnormal biomarker levels in true TCFA negative patients				
		5%	***7.5%***	***10%***	***12.5%***	***15%***
Frequency of abnormal biomarker levels in TCFA positive patients	***10%***	6.1%	6.9%	–	–	–
	15%	7.2%	9.2%	11.1%	13.1%	–
	20%	8.3%	10.3%	12.2%	14.2%	16.1%
	25%	9.4%	11.4%	13.3%	15.3%	17.2%
	30%	10.6%	12.5%	14.4%	16.4%	18.3%
	40%	12.8%	14.7%	16.7%	18.6%	20.6%

Presented data are the expected percentage of patients with abnormal biomarker levels in those who did not have a TCFA in the imaged coronary artery (i.e. *classified* as TCFA negative). The results are displayed for different expected frequencies of abnormal biomarker levels in patients who have a TCFA in the imaged coronary vessel (i.e. TCFA positive) and for different expected frequencies of abnormal biomarker levels in patients who do not have any TCFA in any coronary vessel (i.e. *true* TCFA negative).
TCFA: thin-cap fibroatheroma.

Table 4. Expected statistical power

		Frequency of abnormal biomarker levels in true TCFA negative patients				
		5%	***7.5%***	***10%***	***12.5%***	***15%***
Frequency of abnormal biomarker levels in TCFA positive patients	***10%***	18%	12%	–	–	–
	15%	47%	26%	12%	5%	–
	20%	73%	54%	35%	20%	10%
	25%	88%	76%	61%	44%	29%
	30%	96%	90%	81%	68%	54%
	40%	100%	99%	98%	95%	89%

Presented data are the statistical power to detect differences in the frequency of abnormal biomarker levels between patients with a TCFA in the imaged coronary vessel (i.e. TCFA positive) versus patients without a TCFA in the imaged coronary vessel (i.e. *classified* as TCFA negative) (α-error 5%, two-sided test). The results are displayed for different expected frequencies of abnormal biomarker levels in TCFA positive patients and for different expected frequencies of abnormal biomarker levels in patients who do not have a TCFA in any coronary vessel (*true* TCFA negative). The statistical power is adequate for the most realistic scenarios (grey shaded area).
TCFA: thin-cap fibroatheroma.

Statistical analyses

Conventional linear regression will be applied to relate SNPs, sphingolipids and other biomarkers with IVUS virtual histology measures, corrected for segment length. Mixed linear models will be used for per-lesion analyses. The relation between biomarkers (in a broad sense) and clinical endpoints will be studied by Cox proportional hazard models.

The p-values that appear in the analyses of genetic variants and sphingolipids will be corrected for multiple testing with appropriate methods to adjust for inflation of the type I error (e.g. Bonferroni or simulation). Significant SNPs and lipid fractions need (and will be proposed for) validation in different datasets.

Actual inclusion

A total of 846 patients with complete data for genetic and lipidomics analyses are included in ATHEROREMO-IVUS: 581 patients were enrolled in the Erasmus MC and 265 participated in IBIS-2 (Figure 3).[14] Blood samples are available for 1098 patients. NIRS was performed in 203 patients.

DISCUSSION

The ATHEROREMO-IVUS study was primarily designed to assess correlations of genetic profile and novel circulating biomarkers with the extent, phenotype and vulnerability of coronary atherosclerotic plaques as determined in-vivo by IVUS. Furthermore we

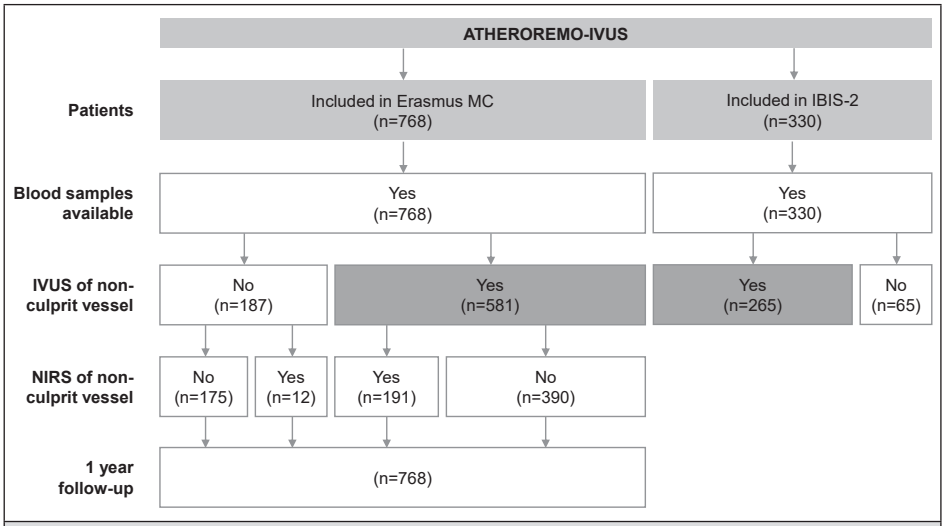

Figure 3. Actual patient inclusion
Finally, blood samples were stored for 1098 patients, IVUS of a non-culprit vessel was performed in 846 patients (grey shaded) and NIRS was performed in 203 patients.
IBIS-2: Integrated Biomarker and Imaging Study-2; IVUS: intravascular ultrasound; NIRS: near-infrared spectroscopy.

would like to assess the potential prognostic value of novel biomarkers, IVUS and NIRS compositional features of atherosclerotic plaques in major cardiac events at long term follow-up.

Acute coronary syndromes are mostly caused by rupture of TCFA lesions that contain a lipid-rich necrotic core covered by a thin fibrous cap.[2,26-29] IVUS virtual histology may be suitable for the detection of such vulnerable plaques. The PROSPECT study has shown that TCFA lesions as identified in-vivo by IVUS virtual histology were associated with increased risk for recurrent cardiovascular events in ACS patients. 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 virtual histology for acute cardiac events as a consequence of spontaneous plaque rupture (i.e. recurrent ACS or death). The prognostic value of IVUS virtual histology in patients with stable angina remains unclear as well. Furthermore, the prognostic value of NIRS for the occurrence of MACE has not yet been investigated. The results of the ATHEROREMO-IVUS study will provide data on these questions.

Coronary artery disease has a strong genetic component. Epidemiological studies suggest that up to 50% of its susceptibility is heritable.[30] Genome wide scans may measure hundreds of thousands of SNPs that can be tested for an association with a coronary atherosclerosis. Although this method is shown to be successful in identifying genetic associations with complex traits,[31] genotyping research programs for atherosclerosis

have been of limited importance so far. One of the bottlenecks was the phenotypic complexity of atherosclerotic vascular diseases. The ATHEROREMO-IVUS study is therefore regarded as a unique opportunity to link genotypes with extensive intracoronary imaging data that reach far beyond the limited knowledge of luminal patency (or stenosis) from conventional coronary angiography.

Several biomarkers of inflammation, coagulation, myocardial necrosis and neurohumoral activation (e.g. C-reactive protein, high-sensitive troponin-T and natriuretic peptides) have more or less been established.[27] Our aim is to explore novel lipid biomarkers in first instance, while validation of more established biomarkers will be done in a later stage of the study.

The design of the ATHEROREMO-IVUS study has several strengths. To our best knowledge, this is the first (large-scale) study to combine several novel intracoronary imaging techniques with extensive genetic analyses, biomarker exploration and validation and adverse clinical outcome during follow-up. Secondly, in this study we examine a single non-culprit vessel. *Ex vivo* as well as *in vivo* studies using IVUS in patients with myocardial infarction have demonstrated the presence of TCFAs in other than the culprit lesion or even culprit artery.[5] Our approach will allow us to test the hypothesis that the phenotype of a non-culprit artery segment (indicating the patient's atherosclerotic disease burden) can be linked to biomarker, genetic and outcome data. If the imaging characteristics of the non-culprit artery appear to be related to the incidence of MACE, then this can be seen as a confirmation that the non-culprit artery reflects atherosclerotic disease burden of the larger coronary vasculature.

Some limitations of this study have to be acknowledged. Firstly, the genetic profile and biomarkers will be correlated with the phenotype of the imaged non-culprit coronary artery only. Although we expect that the presence of TCFA lesions is randomly distributed through the coronary system, we may miss the patient's dominant phenotypic characteristic if this phenotype is only expressed in a coronary segment that has not been imaged (e.g. culprit lesion). Secondly, the ATHEROREMO-IVUS study was designed to explore and discover new genetic and circulating biomarkers. Newly discovered SNPs and lipid biomarkers remain to be validated in another patient cohort.

The results from the ATHEROREMO-IVUS study will improve our understanding on the role of genetic profile and circulating biomarkers in the development of atherosclerosis and vulnerable plaques. Genome wide scans and lipidomics may identify novel biomarkers in coronary artery disease. Furthermore, the prognostic value of novel circulating biomarkers as well as *in-vivo* detection of vulnerable plaques by IVUS virtual histology and NIRS will be assessed. These findings may further contribute to improve risk assessment in patients with coronary artery disease, which may be important for the optimal choice of treatment in the individual patient.

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SUPPLEMENT: DETAILS OF THE ATHEROREMO-NIRS SUBSTUDY

Background

Near-infrared spectroscopy (NIRS) is a novel intracoronary imaging technique that may detect lipid core plaques in the coronary arterial wall.[1-2] Therefore, it may be suitable for in vivo detection of vulnerable plaques. Currently, no data are available on the long-term prognostic value of NIRS in patients with coronary artery disease (CAD). Furthermore associations between NIRS measurements, genetic markers and circulating biomarkers have not been investigated yet. These associations however may further elucidate the pathophysiology of coronary lipid core plaques.

Objectives

The primary objective of the ATHEROREMO-NIRS substudy is to correlate genetic markers and circulating biomarkers with coronary plaque phenotype as determined by NIRS. The secondary objective is to assess the prognostic value of NIRS in patients who underwent coronary angiography for stable angina pectoris or acute coronary syndrome.

Methods

Prior to coronary angiography, a total of 203 patients provided written informed consent for enrollment in the ATHEROREMO-NIRS substudy, in which NIRS imaging was performed in a single, non-stenotic segment of a non-culprit coronary artery. The order of preference for selection of the non-culprit vessel was: 1. left anterior descending artery; 2. right coronary artery artery; 3. left circumflex artery. The FDA-approved NIRS system, as used in this study, consists of a 3.2 French rapid exchange catheter, a pullback and rotation device and a console (InfraReDx, Burlington, Massachusetts, USA). Image acquisition is performed by a motorized catheter pullback at a speed of 0.5mm/s and 240rpm in a proximal segment of a non-culprit artery, starting distal to a side branch. The system performs one thousand chemical measurements per 12.5 mm, in which each measurement interrogates one to two mm^2 of vessel wall from a depth of approximately 1 mm in the direction from the luminal surface towards the adventitia.[1-2] Tissue scattering and absorption of light in the NIR region result in a wavelength dependent return of light to optical detectors that produces a spectrum. Areas of the artery with spectral characteristics of lipid core are displayed as an image map (chemogram) of the studied vessel.

Yellow regions in the chemogram represent high probability for the presence of lipid core-containing coronary plaques (LCP), while red regions represent those with low probability. The x-axis of the chemogram indicates the pullback position in millimeters, while the y-axis indicates the circumferential position of the measurement from zero to 360 degrees. The lipid core burden index (LCBI) score is computed on the basis of the

chemogram by multiplying the fraction of valid yellow pixels by 1.000. Hence, LCBI is a summary measure of the amount of LCP along the entire imaged section of the coronary artery on a 0-to-1000 scale. NIRS images are analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, The Netherlands). Core laboratory personnel are blinded to all other baseline patient, biomarker, genetic and outcome data.

Major adverse cardiovascular events, defined as the composite of all-cause mortality, non-fatal ACS, stroke and unplanned coronary revascularization during 1-year follow-up, were adjudicated by a clinical events committee (CEC) on the basis of original source data. Members of the CEC were blinded to other patient data, NIRS imaging characteristics and genetic or biomarker information.

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CORONARY VULNERABILITY

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NEAR-INFRARED SPECTROSCOPY PREDICTS CARDIOVASCULAR OUTCOME IN PATIENTS WITH CORONARY ARTERY DISEASE

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ABSTRACT

Background: Near-infrared spectroscopy (NIRS) is capable of identifying lipid core-containing plaques, which can subsequently be quantified as a lipid core burden index (LCBI). Currently, no data are available on the long-term prognostic value of NIRS in patients with coronary artery disease (CAD).

Objectives: This study sought to determine the long-term prognostic value of intracoronary NIRS as assessed in a nonculprit vessel in patients with CAD.

Methods: In this prospective, observational study, NIRS imaging was performed in a nonculprit coronary artery in 203 patients referred for angiography due to stable angina pectoris (SAP) or acute coronary syndrome (ACS). The primary endpoint was the composite of all-cause mortality, nonfatal ACS, stroke, and unplanned coronary revascularization.

Results: The 1-year cumulative incidence of the primary endpoint was 10.4%. Cumulative 1-year rates in patients with an LCBI equal to and above the median (43.0) versus those with LCBI values below the median were 16.7% versus 4.0% (adjusted hazard ratio: 4.04; 95% confidence interval: 1.33 to 12.29; p = 0.01). The relation between LCBI and the primary endpoint was similar in SAP and ACS patients (p value for heterogeneity = 0.14). Similar differences between high and low LCBI were observed in pre-specified secondary endpoints.

Conclusion: CAD patients with an LCBI equal to or above the median of 43.0, as assessed by NIRS in a nonculprit coronary artery, had a 4-fold risk of adverse cardiovascular events during 1-year follow-up. This observation warrants confirmation by larger studies with extended follow-up.

INTRODUCTION

Near-infrared spectroscopy (NIRS) is a novel, catheter-based technique capable of identifying lipid core-containing plaques within the coronary artery wall (1). Currently, no data are available on the long-term prognostic value of NIRS in patients with coronary artery disease. We therefore performed a prospective study to assess the prognostic value of coronary plaque detection, as evaluated with NIRS, on the occurrence of major adverse cardiac and cerebrovascular events (MACCE) in the real-world setting of everyday clinical practice, in which patients with both stable angina and acute coronary syndrome (ACS) present for coronary angiography. Parallel to this objective, it was our aim to investigate whether imaging of a single segment without significant luminal narrowing of a nonculprit coronary artery could be used for risk stratification.

METHODS

Study population and design

The ATHEROREMO-NIRS (The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis–Near-Infrared Spectroscopy) substudy (2) was a prospective, single-center, observational study assessing the prognostic value of coronary NIRS, performed at the Thoraxcenter, Erasmus Medical Center, Rotterdam, the Netherlands. All patients had an indication, as determined by their treating physician (as part of routine clinical care), for diagnostic coronary angiography and/or percutaneous coronary intervention (PCI) due to either stable angina pectoris or an ACS. Detailed inclusion and exclusion criteria are listed in Supplemental table 1.

Subsequent to the standard angiography and PCI (when applicable), NIRS of a nonculprit coronary artery was performed. The NIRS target segment of the nonculprit coronary artery was required to be at least 40 mm in length and without significant luminal narrowing (<50% stenosis) as assessed by online angiography. The order of preference for selection of the nonculprit vessels was predefined in the study protocol: 1) left anterior descending artery; 2) right coronary artery; and 3) left circumflex artery. This study was approved by the Medical Ethics Committee of the Erasmus Medical Center, and performed in accordance to the Declaration of Helsinki (2008, 6th revision). Written informed consent was obtained from all participants.

Sample size

The ATHEROREMO-IVUS (The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis–Intravascular Ultrasound) study had a pre-specified sample size of 800 patients, and was designed to explore multiple relations between

genetic and serum biomarkers and coronary plaque characteristics (2). It was during the course of the ATHEROREMO-IVUS study that intracoronary NIRS became commercially available and accessible for our cardiac catheterization lab. The ultimate sample size of the ATHEROREMO-NIRS substudy (203 consecutively consenting patients) was not based on prior effect estimates but rather on the time point of availability and local institutional review board approval (April 2009) of the NIRS technique as ATHEROREMO evolved.

Near-infrared spectroscopy

The U.S. Food and Drug Administration–approved NIRS system, as used in this study, consists of a 3.2-F rapid exchange catheter, a pullback and rotation device, and a console (InfraReDx, Burlington, Massachusetts). Image acquisition was performed by a motorized catheter pullback at a speed of 0.5 mm/s and 240 rpm in a proximal segment of a nonculprit artery, starting distal to a side branch. The system performed 1,000 chemical measurements per 12.5 mm, in which each measurement interrogated 1 to 2 mm^2 of vessel wall from a depth of approximately 1 mm in the direction from the luminal surface toward the adventitia (1). Areas of the artery with spectral characteristics of a lipid core were displayed in yellow within the image map, called a chemogram. NIRS images were analyzed offline by an independent core laboratory (Cardialysis, Rotterdam, the Netherlands). Core laboratory personnel were blinded to all other patient and outcome data.

Study endpoints

The pre-specified primary endpoint was the incidence of MACCE, defined as the composite of all-cause mortality, nonfatal ACS, stroke, and unplanned coronary revascularization during 1-year follow-up, exclusive of events related to the culprit lesion at the index angiography. Secondary endpoints included: 1) the composite of all-cause mortality and nonfatal ACS; 2) the composite of all-cause mortality, nonfatal ACS, and stroke; and 3) the composite of all-cause mortality, nonfatal ACS, and unplanned coronary revascularization during follow-up. Endpoints were adjudicated by a clinical events committee on the basis of original source data. Members of the clinical events committee were blinded to other patient data and NIRS imaging characteristics. Post-discharge survival status was obtained from municipal civil registries. Nonfatal ACS included ST-segment elevation myocardial infarction (STEMI), non-STEMI, or unstable angina pectoris as defined in accordance with the guidelines of the European Society of Cardiology (3,4). Stroke was defined according to the guidelines of the European Stroke Organization (5). Unplanned coronary revascularization was defined as PCI or coronary artery bypass grafting, which initially was not planned after the index angiography and enrollment in the study.

Whenever possible, all events were further adjudicated as related or unrelated to the coronary site that was treated during the index procedure. In case of follow-up

angiography, events were classified either as a definite culprit lesion–related (CLR) event or as related to a coronary site that was not treated during the index procedure (non-CLR event). In case angiographic information on the endpoint related coronary site was not available, the event was classified as indeterminate. The pathophysiological processes of definite CLR events (e.g., in-stent restenosis and stent thrombosis) differ from the pathophysiology of spontaneous plaque rupture. Therefore, data are presented both exclusive of definite CLR events (by default), as well as inclusive of definite CLR events.

Statistical analysis

Normally distributed continuous variables are presented as mean SD. Non-normally distributed continuous variables (e.g., the lipid core burden index [LCBI]) are presented as median (interquartile range [IQR]). Categorical variables are presented in numbers and percentages. Differences in baseline continuous variables between those with an LCBI below versus those equal to and above the median were analyzed by Mann-Whitney U tests, categorical variables by Fisher's exact and Pearson chi-square tests (in case of more than 2 categories). Log or square root transformations were applied whenever homoscedasticity was a required assumption of the used statistical test. Linear regression was used to determine predictors of LCBI. No prior data were ever reported on LCBI distribution of the nonculprit artery. The statistical analytical plan therefore prespecified the median LCBI value as cutoff between "low" and "high" LCBI groups, in case LCBI would appear to be non-normally distributed.

Patients lost to follow-up were considered at risk until the date of last contact, at which time point they were censored. For patients with more than 1 event, the first was counted. Cumulative event rates were estimated according to the Kaplan-Meier method and compared by the log-rank test. Backward stepwise regression analyses were used to determine the predictors of the primary endpoint. Univariable and multivariable Cox proportional hazards regression analyses were applied to evaluate the association between LCBI and 1-year outcome. Three models were used throughout the manuscript: an unadjusted model, an age- and sex-adjusted model, and a "full model." Given the number of events available, adjustment according to a propensity score was used in order to assure parsimony of the full model (6). Variables for the propensity score of the full model were selected on the basis of clinical relevance and significance after backward stepwise regression. The propensity scores were derived from predicted probabilities in logistic regression models with LCBI above the median as dependent variable (7). The propensity score that was entered into the full model accounts for age, sex, hypercholesterolemia, diabetes, hypertension, indication for index coronary angiography (stable angina pectoris versus ACS), history of myocardial infarction, peripheral artery disease (PAD) or stroke, and prior PCI. Crude and adjusted hazard ratios (HRs) are presented with 95% confidence intervals (CIs). Discrimination of the full model with respect to event

prediction was evaluated with receiver-operating characteristic curves. Heterogeneity of the effect of LCBI on MACCE was tested between patients presenting with stable angina and those presenting with ACS at the time of enrollment (8). All statistical tests were 2-sided with a type I error level of 0.05. Analyses were performed with IBM SPSS statistics version 20.0 (IBM Corp., Armonk, New York).

RESULTS

Between April 16, 2009, and January 28, 2011, a total of 203 patients were enrolled prior to coronary angiography. Median follow-up was 1 year and follow-up data were complete in 100% of the study sample. Mean age was 63.4 years. Men constituted 72.9% of the study sample and 46.8% of the patients presented with an ACS. A PCI was performed in 88.2% of the patients during the index coronary angiography.

LCBI values in the nonculprit vessel (median pullback length: 63.1 mm; IQR: 51.0 to 75.0 mm) ranged from 0 to 571, with a median of 43.0 (IQR: 15.0 to 90.0) (Figure 1). Regression analysis demonstrated that men and patients with a history of hypercholesterolemia, stroke, or PAD had higher LCBI values. Differences in baseline characteristics between patients with an LCBI below the median versus those with an LCBI equal to or above the median value are presented in Table 1. LCBI of the nonculprit imaged segment did not differ between patients presenting with stable angina (median: 35.0; IQR: 14.0 to 85.5) or ACS (median: 47.0; IQR: 16.0 to 90.0; p = 0.24).

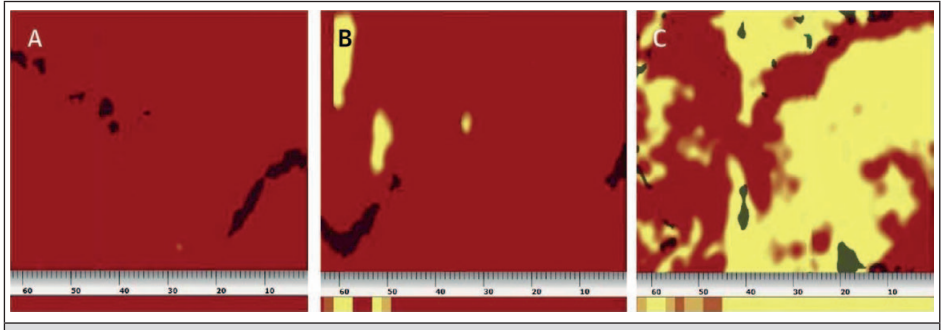

Figure 1. Intracoronary near-infrared spectroscopy displayed as a chemogram
The figures display the graphical result of coronary wall evaluation with near-infrared spectroscopy in 3 different patients. Spectral characteristics of lipid core– containing coronary plaques (LCP) are displayed along the length (x-axis, in mm) and circumference (y-axis, 0 to 360) of the scanned coronary artery. Yellow regions in the chemogram represent high probability for the presence of LCP, while red regions represent those with low probability. The lipid core burden index (LCBI) score is computed on the basis of the chemogram by multiplying the fraction of valid yellow pixels within the region of interest by 1,000. The LCBI for the different patients depicted are 0 (A), 43 (B), and 571 (C) (examples of the lowest, median, and highest value in our study, respectively).

Table 1. Baseline characteristics.

	All patients N = 203	LCBI < Median N= 101	LCBI ≥ Median N=102	P value
Patient characteristics				
Age, years	63.4 ±10.9	64.8 ±10.8	62.1 ±11.0	0.083
Male, n (%)	148 (72.9)	67 (66.3)	81 (79.4)	0.041
Diabetes Mellitus, n (%)	41 (20.2)	18 (17.8)	23 (22.5)	0.485
Hypertension, n (%)	114 (56.2)	56 (55.4)	58 (56.9)	0.888
Hypercholesterolemia, n (%)	115 (56.7)	53 (52.5)	62 (60.8)	0.259
Smoking, n (%)	50 (24.6)	23 (22.8)	27 (26.5)	0.805
Positive family history, n (%)	120 (59.1)	62 (61.4)	58 (57.4)	0.667
Previous MI, n (%)	79 (38.9)	36 (35.6)	43 (42.2)	0.389
Previous PCI, n (%)	78 (38.4)	39 (35.6)	39 (38.2)	1.000
Previous CABG, n (%)	6 (3.0)	4 (4.0)	2 (2.0)	0.445
Previous stroke, n (%)	6 (3.0)	1 (1.0)	5 (4.9)	0.212
Peripheral artery disease, n (%)	11 (5.4)	4 (4.0)	7 (6.9)	0.537
History of renal insufficiency, n (%)	12 (5.9)	5 (5.0)	7 (6.9)	0.767
History of heart failure, n(%)	9 (4.4)	6 (5.9)	3 (2.9)	0.331
Statin at discharge	181 (89.2)	91 (90.1)	90 (88.2)	0.82
Median total cholesterol (IQR)	4.20 (3.60-5.20)	4.20 (3.40-5.00)	4.30 (3.68-5.30)	0.301
Median low-density lipoprotein (IQR)	2.47 (1.95-3.21)	2.44 (1.85-3.14)	2.49 (2.03-3.39)	0.381
Median high-density lipoprotein (IQR)	1.14 (0.92-1.36)	1.15 (0.93-1.37)	1.09 (0.92-1.32)	0.455
Median triglycerides (IQR)	1.26 (0.91-1.80)	1.18 (0.89-1.73)	1.35 (0.95-1.91)	0.152
Procedural characteristics				
Indication for coronary angiography				0.261
ACS	95 (46.8)	43 (42.6)	52 (51.0)	
Acute MI, n (%)	67 (33.0)	15 (14.9)	13 (12.7)	
Unstable angina, n (%)	28 (13.8)	28 (27.7)	39 (38.2)	
Stable angina	108 (53.2)	58 (57.4)	50 (49.0)	
PCI / stent implantation	179 (88.2)	86 (85.1)	93 (91.2)	0.199
Extent of coronary artery disease				0.045
No significant stenosis, n (%)	16 (7.9)	10 (9.9)	6 (5.9)	
1-vessel disease, n (%)	106 (52.2)	58 (57.4)	48 (47.1)	
2-vessel disease, n (%)	58 (28.6)	28 (27.7)	30 (29.4)	
3-vessel disease, n (%)	23 (11.3)	5 (5.0)	18 (17.6)	
NIRS characteristics				
Imaged coronary artery				0.299
Left anterior descending, n (%)	74 (36.5)	42 (41.6)	32 (31.4)	
Left circumflex, n (%)	70 (34.5)	31 (30.7)	39 (38.2)	
Right coronary artery, n (%)	59 (29.1)	28 (27.7)	31 (30.4)	
Median LCBI (IQR)	43.0 (15.0-90.0)	15.0 (6.0-27.0)	88.5 (58.8-120.3)	<0.001

LCBI, Lipid Core Burden Index; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; IQR, interquartile range, ACS, acute coronary syndrome.

Table 2. Clinical event distribution during one-year follow-up.

	All-cause mortality	Non-fatal ACS	Stroke	Unplanned coronary revascularization	All events (incl. CLR events)	All events (excl. CLR events)
Primary endpoint, *n (%)*						
All-cause mortality, non-fatal ACS, stroke and unplanned coronary revascularization	7 (3.4)	6 (3.0)	3 (1.5)	12 (5.9)	**28 (13.8)**	**21 (10.3)**
Secondary endpoint, *n (%)*						
All-cause mortality and non-fatal ACS	7 (3.4)	6 (3.0)	n.a.	n.a.	**13 (6.4)**	**10 (4.9)**
All-cause mortality, non-fatal ACS and stroke	7 (3.4)	6 (3.0)	3 (1.5)	n.a.	**16 (7.8)**	**13 (6.4)**
All-cause mortality, non-fatal ACS and unplanned coronary revascularization	7 (3.4)	6 (3.0)	n.a.	12 (5.9)	**25 (12.3)**	**18 (8.9)**

Percentages are given for the cumulative incidence. ACS, acute coronary syndrome; CLR events, culprit lesion-related events.

MACCE occurred in 28 patients (13.8%) during 1-year follow-up. Seven events (25% of the total number of events) were classified as definite CLR events, hence the primary endpoint (which excludes definite CLR events) occurred in 21 patients. The 1-year cumulative incidence of the primary endpoint was 10.4%. The frequencies of all first events are described in Table 2. Unplanned coronary revascularization (5.9%; all events were revascularized by PCI) occurred most frequently, followed by all-cause mortality (3.4%), nonfatal ACS (3.0%), and stroke (1.5%). Only LCBI, a history of stroke, and PAD were associated with the primary endpoint.

The 1-year cumulative rate of the primary endpoint was 16.7% for patients with an LCBI equal to and above the median versus 4.0% for those with LCBI values below the median (log-rank p = 0.003) (Figure 2) (unadjusted HR: 4.56; 95% CI: 1.53 to 13.55). The secondary endpoint of all-cause mortality and nonfatal ACS occurred in 8.8% versus 1.0% in those with high versus low LCBI (p = 0.010) (Figure 2). Cumulative event rates for other secondary endpoints – also exclusive of definite CLR events – also are shown in Figure 2.

The association between LCBI equal to and above the median value and the primary endpoint remained significant after adjustment for age and sex (adjusted HR: 5.16; 95% CI: 1.73 to 15.42) and after adjustment for the full model (adjusted HR: 4.04; 95% CI: 1.33 to 12.29), as described in Table 3. LCBI values equal to and above the median were significantly associated with an increased risk of all secondary endpoints with point estimates of the (full model) adjusted HRs ranging from 3.56 to 10.59 (Table 3). With

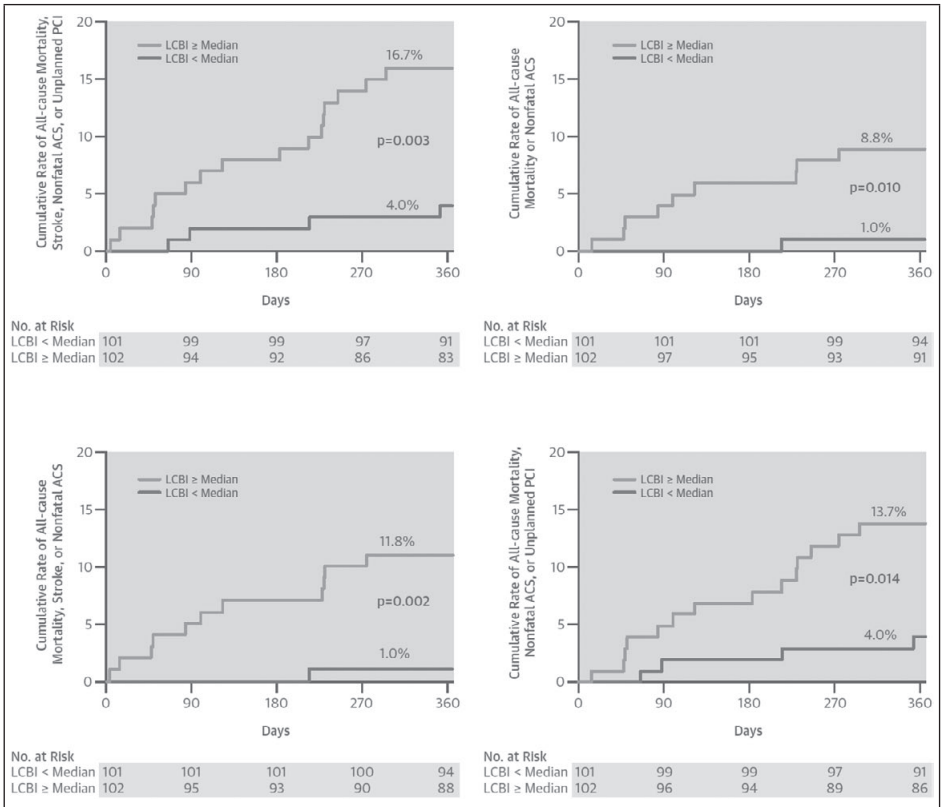

Figure 2. Time-to-event curves for the primary and pre-specified secondary composite endpoints exclusive of definite culprit lesion-related events

A 4-fold increase in major adverse cardiac and cerebrovascular events during 1-year follow-up was observed in patients with a lipid core burden index (LCBI) above the median, as assessed by near-infrared spectroscopy of a nonstenotic nonculprit coronary artery segment (16.7% vs. 4.0% event rate [adjusted hazard ratio: 4.04; 95% confidence interval: 1.33 to 12.29; p = 0.01]). ACS = acute coronary syndrome; PCI = percutaneous coronary intervention.

respect to prediction of the primary endpoint, the full model resulted in an area under the receiver-operating characteristic curve of 0.83 (95% CI: 0.75 to 0.92).

There was a statistically significant difference in mortality between those below and above the median in univariate analysis (1.0% vs. 6.9%; log-rank p = 0.032), but not after adjusting for the full model (adjusted HR: 6.2; 95% CI: 0.73 to 52.0; p = 0.10).

The median LCBI in patients with stable coronary artery disease was 35.0 (IQR: 14.0 to 85.5) and did not differ significantly from the median of 47.0 (IQR: 16.0 to 90.0) in patients with ACS at index angiography (p = 0.44). We found no heterogeneity of the effect of LCBI on the primary endpoint between patients presenting with stable angina versus ACS patients at the time of enrollment (p value for heterogeneity = 0.14).

Table 3. LCBI levels equal to and above the median value and major adverse cardiac events (exclusive of definite culprit lesion-related events).

	Unadjusted model	P	Age and gender adjusted model	P	Full model	P
Primary endpoint	Hazard ratio (95% CI)	**value**	Hazard ratio (95% CI)	**value**	Hazard ratio (95% CI)	**value**
All-cause mortality, non-fatal ACS, stroke and unplanned coronary revascularization	4.56 (1.53-13.55)	0.006	5.16 (1.73-15.42)	0.003	4.04 (1.33-12.29)	0.014
Secondary endpoints						
All-cause mortality and non-fatal ACS	9.36 (1.19-73.87)	0.034	10.14 (1.27-80.67)	0.029	8.91 (1.10-72.33)	0.041
All-cause mortality, non-fatal ACS and stroke	12.67 (1.65-97.46)	0.015	14.58 (1.89-112.71)	0.010	10.59 (1.35-83.28)	0.025
All-cause mortality, non-fatal ACS and unplanned coronary revascularization	3.69 (1.21-11.20)	0.021	3.96 (1.29-12.11)	0.016	3.56 (1.14-11.20)	0.029

* Hazard ratios are given for patients with lipid core burden index (LCBI) levels equal to and above the median (n=102), versus those with LCBI below the median (n=101). Variables in the propensity score of the full model were age, gender, hypercholesterolemia, diabetes, hypertension, history of myocardial infarction, peripheral artery disease and stroke, indication for index coronary angiography (stable angina versus ACS) and prior PCI. ACS, acute coronary syndrome.

Analyses inclusive of definite culprit lesion-related events

Unplanned PCI was required for 4 culprit lesions that had been treated during the index catheterization; in 3 other patients, initially treated culprit lesions led to unstable angina, non-STEMI, and death, respectively.

When definite culprit lesion-related events were also taken into account, an overall 1-year cumulative rate of the primary endpoint was observed in 19.6% of the patients with an LCBI equal to and above the median versus 7.9% for those with LCBI values below the median (p = 0.015) (Supplemental Figure 1).

The secondary endpoint of all-cause mortality and nonfatal ACS occurred in 9.8% versus 3.0% in those with high versus low LCBI, respectively (p = 0.010). Similar results were observed for the other 2 secondary endpoints.

DISCUSSION

We observed that high LCBI levels were associated with a 4-fold increase in MAC(C)E during 1-year follow-up of a broad population of patients referred for coronary angiography.

This association between LCBI and adverse outcome was found by NIRS imaging of a single, nonstenotic segment of a nonculprit coronary artery.

Based on diffuse reflectance spectroscopy, NIRS provides a positive and specific chemical measure of cholesterol within the coronary vessel wall, as cholesterol has prominent molecular features in the near-infrared region that can be distinguished from other tissue constituents such as collagen (9). Its ability to recognize cholesterol monohydrate and cholesterol ester – both of which are abundant in necrotic cores and therefore key components of plaque vulnerability – appears to be superior to that of intravascular ultrasound (IVUS) – or optical coherence tomography-based techniques (10).

Against this background, the recently published, randomized YELLOW (Reduction in Yellow Plaque by Aggressive Lipid-Lowering Therapy) trial is of particular interest, as it is the first study to investigate whether a pharmacologic intervention may reduce lipid core plaque as assessed by intracoronary NIRS (11). High-dose statin therapy (vs. standard-of-care statin therapy) during 6 to 8 weeks in 87 patients resulted in a significant reduction of LCBI as measured at the site of untreated obstructive coronary lesions with a fractional flow reserve below 0.80 (11). It is important to emphasize, however, that the association between LCBI and MAC(C)E in our study was found through imaging of a segment of a nonculprit coronary artery without significant stenosis. Consequently, the median LCBI of 43.0 (IQR: 15.0 to 90.0) in our study is lower than the median LCBI values of 95.4 (IQR: 29.6 to 174.6) and 132.4 (IQR: 99.0 to 201.2), for the standard-of-care and high-dose statin therapy groups, respectively, as measured in the obstructive lesions in the YELLOW trial (11). The prognostic value of NIRS imaging of such obstructive lesions has not yet been investigated for risk prediction of adverse cardiovascular events during long-term follow-up.

The currently ongoing IBIS-3 (Integrated Biomarker and Imaging Study-3) study is designed to assess the efficacy of high-dose rosuvastatin on the reduction of the necrotic core and LCBI in a nonculprit coronary segment of patients who have undergone diagnostic angiography or PCI (12).

Our data were prospectively obtained and the conclusions seem applicable to a broad range of patients, including those with stable angina or ACS at the time of index angiography. Of great importance is that NIRS evaluation was performed in an independent, dedicated core lab with personnel blinded for patient and outcome data. An independent and blinded clinical event committee adjudicated the events. Statistics were performed by authors who were not, in any way, involved with the study until the time of transfer of the finalized database.

Study limitations

There are several limitations to our findings. The small sample size and corresponding number of events are a limitation, although this study does represent the largest cohort of patients with NIRS analysis and long-term follow-up so far.

Furthermore, the ATHEROREMO-NIRS substudy was a single-center study by virtue of design. External validation, preferably in a larger sample size, is a fundamental prerequisite before any of our conclusions may be considered for possible future clinical implications.

Our cutoff value was based on the median LCBI value, similarly to a recent post hoc analysis of the SATURN (Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin vs. Atorvastatin) trial, which evaluated the median value of IVUS-derived percent atheroma volume as cutoff for predicting future MAC(C)E (13). We do not propose 43 as an absolute cutoff value. Larger sample sizes are required to determine and validate the sensitivity and specificity of NIRS imaging at different cutoff LCBI values.

Accordingly, future research will have to demonstrate whether the strength of NIRS is determined by its capability to detect or to rule out an increased risk for MAC(C)E during long-term follow-up.

Previous studies with IVUS have repeatedly demonstrated that IVUS-derived plaque volume in comparable nonstenotic, nonculprit coronary segments was associated with incident MAC(C)E during longterm follow-up (13,14). Similarly, the ATHEROREMO protocol proposed NIRS imaging in at least 4 cm (median pullback length: 63.1 mm) of only 1 proximal segment of a nonculprit coronary artery. Nevertheless, it should be emphasized that our findings relate to an increased risk of MAC(C)E throughout the entire coronary tree and not necessarily at the imaged segment or a lesion-specific risk. We did not aim to identify all "vulnerable" and potentially treatable plaques. Our study does not allow conclusions on whether the events during follow-up originated from regions of relatively high or low LCBI or whether more proximal cholesterol accumulation is associated with an increased event rate. Three-vessel NIRS imaging at index angiography and follow-up coronary angiography (or autopsy) at the moment of an endpoint are ideally required for such conclusions.

From an etiologic point of view, it is important to emphasize that the specificity of in vivo detection of potentially vulnerable plaques and knowledge about their temporal stability generally is limited given the current state of the art (15). As we did not repeat NIRS imaging of the same segment at a later time point, no conclusions can be drawn on temporal plaque stability and dynamics. Rather than an etiologic exploration to identify all coronary plaques and assess their temporal stability, our analyses should be seen as an evaluation of the prognostic value of NIRS imaging used as a global marker of intracoronary disease burden, which is not seen in the form of luminal narrowing on angiography.

The majority of the endpoints in this study were due to unplanned revascularization. Future studies with a higher incidence of mortality and nonfatal ACS are required to properly assess the prognostic value of NIRS for these events.

Formal testing demonstrated that there was no heterogeneity of the ability of LCBI to predict outcome between the stable angina and ACS groups, although the interaction test may have been underpowered.

Intracoronary NIRS became commercially available during the course of ATHEROREMO. Thus, understandably, we were not able to enroll all ATHEROREMO patients in this NIRS substudy. The characteristics, treatment, and outcomes of the substudy cohort and the remaining ATHEROREMO patients were similar. Hence, differential selection is unlikely, although it cannot be excluded with absolute certainty.

A limitation of the NIRS technique is that the chemograms only provide plaque information in a 2-dimensional manner and do not provide information on the depth of the cholesterol accumulation within the coronary artery wall. IVUS may therefore be used for additional evaluation of luminal stenosis, vessel remodeling, and plaque architecture.

CONCLUSIONS

This prospective observational study suggests that coronary LCBI, as assessed by NIRS in a nonculprit coronary artery, is associated with MAC(C)E during 1-year follow-up in patients referred for coronary angiography. However, our results are hypothesis generating and need confirmation by larger trials that overcome the limitations of our study.

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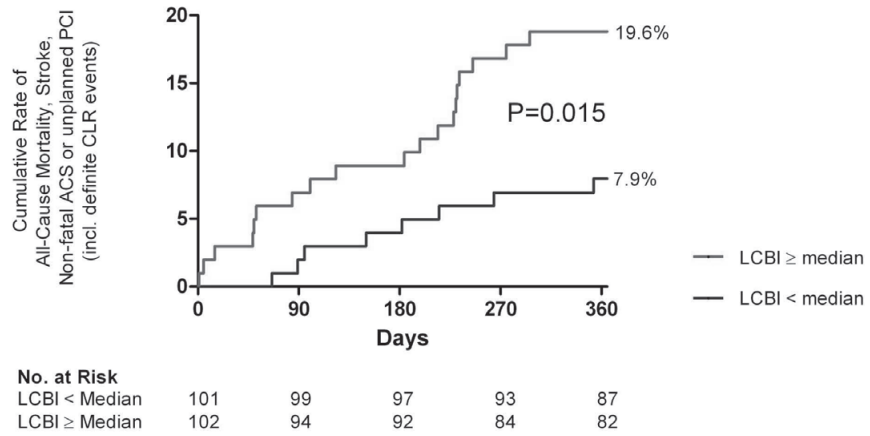
SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1. Inclusion and exclusion criteria

Inclusion criteria:
8. Aged 21 years or older. 9. Presenting with stable angina pectoris (CCS angina class 1, 2, 3 or 4), unstable angina pectoris (Braunwald class 1-3, B-C), documented silent ischemia or acute myocardial infarction (STEMI and NSTEMI). 10. Eligible for coronary revascularization in the native coronary artery/arteries. 11. Willing and able to comply with the specified follow-up evaluation. 12. Willing to sign informed consent. 13. Presence of a flow-limiting stenosis (diameter stenosis ≥50% by QCA or visual estimate) that is held responsible for angina pectoris or acute coronary syndrome 14. The study vessel has not undergone percutaneous coronary intervention in the last 8 months.
Exclusion criteria:
17. Angina caused by a non-cardiac illness (Braunwald class IA, IIA, IIIA). 18. Pregnant women or women of childbearing potential who do not use adequate contraception. 19. Known allergies to aspirin, clopidogrel, ticlopdine, heparin, stainless steel, copper or a sensitivity to contrast media which cannot be adequately pre-medicated. 20. Previous participation in this study or participation in another study with any investigational drug or device within the past 30 days (study participation ends after completion of the final follow-up). 21. Life expectancy of less than one year or factors making clinical and/or angiographic follow-up difficult. 22. Planned or being status post coronary bypass surgery. 23. Planned major non-cardiac surgery. 24. Impaired renal function (creatinine>2 mg/dl or ≥150 µmol/l). 25. History of bleeding diathesis or coagulopathy. 26. History of disabling stroke within the past year.
Exclusion criteria for intravascular ultrasound and near-infrared spectroscopy:
27. Three-vessel coronary artery disease or left main disease with ≥50% stenosis. 28. Minimal lumen diameter <2mm in the segments to be analyzed within the study vessel. 29. Diameter stenosis >70% or total occlusion of the study vessel. 30. In case the study-vessel has been stented previously (>8 months ago), more than 1/3 proximal of the study vessel (at least 40mm in length) should be available for examination (i.e. outside the length of the stent plus 5mm proximal to the stent). 31. Poor left ventricular function as assessed by echocardiography or by angiography. 32. Moderate or severe tortuosity of the study segment (i.e. 2 bends >75° or one bend > 90°). 17. Known tendency for coronary vasospasm.

CCS: Canadian Cardiovascular Society; NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; QCA: quantitative coronary angiography.



Supplemental Figure 1. Time-to-Event curves for the composite of all-cause mortality, stroke, non-fatal ACS or unplanned PCI, including definite culprit lesion-related events, during one-year follow-up of 203 patients.

The cumulative event rate applies to the analysis in which all 28 events, including the definite culprit lesion-related events, were assessed. LCBI = Lipid Core Burden Index; ACS = acute coronary syndrome; PCI = percutaneous coronary intervention; CLR events = culprit lesion-related events.

CORONARY VULNERABILITY

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NEAR-INFRARED SPECTROSCOPY-DERIVED LIPID CORE BURDEN INDEX PREDICTS ADVERSE CARDIOVASCULAR OUTCOME IN PATIENTS WITH CORONARY ARTERY DISEASE DURING LONG-TERM FOLLOW-UP

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ABSTRACT

Aims: Near-infrared spectroscopy (NIRS) is able to quantify cholesterol within coronary arteries by the lipid core burden index (LCBI). We studied the prognostic value of NIRS-derived LCBI in patients with coronary artery disease (CAD) for adverse cardiac outcome during long-term follow-up.

Methods and results: During 2009-2013, NIRS was performed in a non-culprit artery of 275 patients undergoing coronary angiography for acute coronary syndrome (ACS) or stable angina. LCBI was quantified by an independent corelab for the region of interest ($LCBI_{ROI}$) and the 4 and 10 mm long segment with the maximum LCBI ($MaxLCBI_{4mm}$ and $MaxLCBI_{10mm}$). The primary endpoint was major adverse cardiac events (MACE), defined as the composite of all-cause death, non-fatal ACS, or unplanned revascularization. Hazard ratios (HR) were adjusted for age, gender, clinical risk factors and segment plaque burden based on intravascular ultrasound. During a median follow-up of 4.1 years, 79 patients (28.7%) had MACE. There was a statistically significant and independent continuous relationship between higher $MaxLCBI_{4mm}$ values and a higher risk of MACE. Each 100 units increase of $MaxLCBI_{4mm}$ was associated with a 19% increase in MACE (HR 1.19, 95%CI:1.07-1.32, p=0.001). Continuous $MaxLCBI_{4mm}$ remained independently associated with MACE after exclusion of target lesion-related events (HR 1.21, 95CI%:1.08-1.35), as well as after exclusion of adverse events related to the NIRS-imaged coronary segment (HR 1.19, 95%CI:1.06-1.34). Results for $MaxLCBI_{10mm}$ were comparable.

Conclusion: NIRS-derived LCBI is associated with adverse cardiac outcome in CAD patients during long-term follow-up independent of clinical risk factors and plaque burden.

INTRODUCTION

Coronary artery disease (CAD) is projected to remain the leading cause of mortality and morbidity worldwide. Patients with a history of CAD are at higher risk of subsequent adverse cardiovascular events, such as an acute coronary syndrome (ACS). In approximately 75% of all cases, an ACS is caused by rupture or fissure of a vulnerable, lipid rich core-containing plaque in the coronary arteries.[1,2] While coronary angiography (CAG) is unable to identify such lipid rich core-containing plaques in the coronary artery wall,[3] they can be identified by near-infrared spectroscopy (NIRS), a catheter-based intracoronary imaging technique based on diffuse reflectance spectroscopy.[4-6] Therefore, NIRS may be useful in identifying patients at increased risk of adverse cardiovascular outcome.[5-7]

The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis (ATHEROREMO) and the Integrated Biomarker Imaging Study 3(IBIS-3) studies were designed to investigate phenotypes and vulnerability of coronary atherosclerosis as determined by intravascular ultrasound (IVUS) and NIRS.[8,9] NIRS became available in our cardiac catheterization laboratory during the course of both the ATHEROREMO and IBIS-3 study.[10] In the current study, we performed long-term follow-up of both the ATHEROREMO-NIRS and IBIS-3-NIRS substudies, with the aim to investigate the long-term prognostic value of lipid rich core-containing plaques as assessed by NIRS in patients with CAD undergoing CAG.

METHODS

Study design and population

The current investigation combines the populations of the ATHEROREMO-NIRS and the IBIS-3-NIRS substudies. Both of these studies were conducted at the Erasmus Medical Center, Rotterdam, The Netherlands, and had similar enrollment criteria and baseline study procedures. The study designs and methods of ATHEROREMO-NIRS and IBIS-3-NIRS have been described in detail elsewhere.[8-10] Briefly, patients undergoing diagnostic CAG or PCI for ACS or stable angina pectoris (SAP) underwent baseline invasive imaging by NIRS and IVUS, and were subsequently followed-up on adverse cardiovascular events.[11,12] The obtained images were analyzed off-line, and findings were not used for patient care. In ATHEROREMO-NIRS, patient management was left to the discretion of the treating physician. In IBIS-3, as per protocol, high-dose rosuvastatin was prescribed during the first year after the index event. ATHEROREMO-NIRS enrolled 203 patients between April 2009 and January 2011, and IBIS-3-NIRS enrolled 131 patients between January 2010 and June 2013. Since 48 patients participated in both studies, a total of

286 patients were available. Of these patients, 275 patients had baseline data available on both NIRS and IVUS, and were therefore included in the current analysis.

The medical ethics committee of the Erasmus MC approved both the ATHEROREMO-NIRS and IBIS-3-NIRS substudy. These two studies were performed in accordance with the declaration of Helsinki. All patients provided written informed consent for their participation and for compliance with the study protocols, including long-term follow-up. The ATHEROREMO study is registered in ClinicalTrials.gov, number NCT01789411, and the IBIS-3 study is registered in The Netherlands trial register, number NTR2872.

Near-infrared spectroscopy

Subsequent to the standard index CAG, invasive imaging with IVUS and NIRS was performed in a non-culprit coronary artery. The NIRS target segment in this non-culprit coronary artery was required to be at least 40 mm in length and without significant luminal narrowing (<50% stenosis) as assessed by on-line angiography. The study protocol predefined the order of preference for the selection of the non-culprit vessel.[8,9]

The NIRS system included a 3.2-F rapid exchange catheter, a console and a rotation and pullback device (InfraRedx, Burlington, Massachusetts). Images were acquired by the NIRS catheter that was automatically pulled back at a speed of 0.5 mm/s and 240 rotations per minute in a proximal segment of the non-culprit artery, as described in detail previously.[5,10] The fraction of yellow pixels obtained from the chemogram, an image map derived from the NIRS measurements, was multiplied by 1000 to compute the Lipid Core Burden Index (LCBI). Therefore, the 4 mm long segment with the maximum LCBI ($MaxLCBI_{4mm}$) ranged from 0 to 1000 representing the percentage of lipid core in the investigated segment.[6] Moreover, the 10 mm long segment with the maximum LCBI ($MaxLCBI_{10mm}$) was quantified, and the same was done for the region of interest ($LCBI_{ROI}$) of the investigated segment. NIRS data were analyzed off-line by an independent corelab (Cardialysis, Rotterdam, The Netherlands) blinded to all other patient and outcome data.

Intravascular ultrasound

After the standard index CAG, the non-culprit segment was first examined by IVUS. IVUS images were acquired by the Volcano Eagle Eye Gold IVUS catheter (20 MHz).[8] Analyses of the IVUS gray-scale data were performed using the pcVH 2.1 and qVH software (Volcano Corp., San Diego, CA, USA). Segmental plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area.[8] IVUS gray-scale data were also analyzed off-line.

Study endpoints

The primary endpoint consisted of major adverse cardiac events (MACE), defined as the composite of all-cause death, non-fatal ACS, or unplanned coronary revascularization

during long-term follow-up. A secondary analysis was performed on the composite endpoint of cardiac death, non-fatal ACS, or unplanned revascularization. Furthermore, additional analyses were performed on these two endpoints after exclusion of definite target lesion-related events, as well as after exclusion of adverse events related to the NIRS-imaged coronary segment.

Follow-up was conducted in January 2015. Vital status of the patients was obtained from municipal civil registries. Follow-up questionnaires were subsequently sent to all living patients as a first screening method for identifying possible adverse events. Thereafter, hospital discharge letters were obtained if any hospitalization or possible event was reported. In patients who did not return the questionnaire, the local hospital records were investigated for possible events. Cause of death was obtained from hospital records, autopsy reports or general practitioners notes.

MACE were adjudicated based on original source data by a clinical events committee blinded to patient characteristics and NIRS and IVUS data. In accordance with the guidelines of the European Society of Cardiology, non-fatal ACS was defined as the clinical diagnosis of ST-segment Elevation Myocardial Infarction (STEMI), non-STEMI (NSTEMI), or unstable angina pectoris.[13,14] Unplanned coronary revascularization was defined as any PCI or coronary artery bypass grafting (CABG) that was not planned after the index angiography and enrollment in the study. Cardiac death was defined as any death due to proximate cardiac cause, unwitnessed death or death of unknown cause.

Furthermore, the clinical event committee adjudicated whether the cardiac events were related to the target lesion that was treated during the index procedure, as well as whether the events were related to the coronary artery segment that was imaged at baseline.

Statistical analysis

Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Normally-distributed continuous variables were reported as means and standard deviations. Non-normally-distributed continuous variables were reported as medians and interquartile ranges (IQR), categorical variables as numbers and percentages.

Patients that were lost to follow-up were censored at the date of last contact. The first event was considered in case a patient had multiple events. The Kaplan-Meier method was used to estimate cumulative event rate. All subsequent analyses were performed for each of the three LCBI variables. The log-rank test was used to compare cumulative event rates between quartiles of the LCBI variables and pairwise comparisons were performed when the overall log-rank test showed statistical significant differences.

The association between LCBI and the long-term incidence of study endpoints was analyzed by Cox proportional hazard regression analyses. Furthermore, to evaluate whether the association between LCBI and log(hazard) was linear enough to fit as a single

degree of freedom regression term, a spline was inserted in each full Cox proportional hazard regression model and visual inspection of the estimated relation was performed. No evidence was found for non-linearity with respect to $MaxLCBI_{4mm}$, whereas findings with respect to $MaxLCBI_{10mm}$ and $LCBI_{ROI}$ were borderline significant. Visual inspection of the estimated relation showed that categorization of LCBI in quartiles resulted in an acceptable piece-wise linearity for all endpoints. For Cox regression analyses, consecutively, unadjusted models and multivariable models containing clinical characteristics and IVUS derived plaque burden were used. Potential confounders were chosen based on existing literature. The multivariable models contained the following potential confounders: age, gender, indication for index CAG (ACS or SAP), diabetes mellitus, history of cerebrovascular accident, history of peripheral artery disease and IVUS derived segmental plaque burden. Hazard ratios (HRs) were reported with 95% confidence intervals (CIs). Although this study did not aim to develop a prognostic model per se, a C-index was reported for each multivariable model to provide some indication of the prognostic value of continuous LCBI in addition to clinical risk factors and plaque burden.

All statistical tests were two-tailed and p-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS statistics version 21.0 (IBM Corp., Armonk, New York).

RESULTS

Baseline characteristics

Mean age of the patients was 62.5 years and 76.7% were men (Table 1). A total of 42.5% of the patients presented with an ACS. $MaxLCBI_{4mm}$ values in the non-culprit artery ranged from 0 to 930, with a median of 227.0 (IQR:83.0-360.0). The $LCBI_{ROI}$ values ranged from 0 to 571, with a median of 40.0 (IQR:13.0-79.0). PCI was performed in 88.4% of the patients during the index procedure.

Incidence of primary endpoint

Median follow-up time was 4.1 (IQR:3.2-4.5) years. The follow-up questionnaire assessing the occurrence of MACE was completed by 90% of the patients. The primary composite endpoint of all-cause death, non-fatal ACS or unplanned revascularization occurred in 79 patients (28.7%). All-cause death occurred in 20 patients, non-fatal ACS in 40 patients and unplanned revascularization in 62 patients. The composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization occurred in 70 patients (25.5%).

Table 1. Baseline characteristics	
	N=275 patients
Clinical characteristics	
Age, years	62.5 ± 10.7
Men, n(%)	211 (76.7)
Diabetes, n(%)	59 (21.5)
Hypertension, n(%)	165 (60.0)
Dyslipidemia, n(%)	158 (57.5)
Current smoking, n(%)	69 (25.1)
Previous MI, n(%)	94 (34.2)
Previous PCI, n(%)	98 (35.6)
Previous CABG, n(%)	6 (2.2)
Previous CVA, n(%)	16 (5.8)
History of PAD, n(%)	15 (5.5)
History of renal impairment, n(%)	14 (5.1)
Laboratory measurements, (mmol/l)	
Median total cholesterol (IQR)	4.10 [3.60-5.00]
Median low-density lipoprotein (IQR)	2.42 [1.93-3.13]
Median high-density lipoprotein (IQR)	1.14 [0.92-1.35]
Procedural characteristics	
Indication for coronary angiography	
ACS, n(%)	117 (42.5)
Acute MI, n(%)	31 (11.3)
Unstable angina, n(%)	86 (31.3)
Stable angina, n(%)	158 (57.5)
PCI performed in non-imaged vessel, n(%)	243 (88.4)
Coronary artery disease	
No significant stenosis, n(%)	18 (6.5)
1-vessel disease, n(%)	144 (52.4)
2-vessel disease, n(%)	87 (31.6)
3-vessel disease, n(%)	26 (9.5)
NIRS characteristics	
Imaged coronary artery	
Left anterior descending, n(%)	96 (34.9)
Left circumflex, n(%)	97 (35.3)
Right coronary artery, n(%)	82 (29.8)
Median imaged segment length, mm (IQR)	56.4 [45.3-67.2]
Median $LCBI_{ROI}$ (IQR)	40.0 [13.0-79.0]
Median $MaxLCBI_{10mm}$ (IQR)	129.0 [48.0-234.0]
Median $MaxLCBI_{4mm}$ (IQR)	227.0 [83.0-360.0]
IVUS derived Segment Plaque Burden (%)	39.3 ± 11.0

MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary bypass grafting; CVA, cerebrovascular accident; PAD, peripheral artery disease; ACS, acute coronary syndrome; NIRS, near-infrared spectroscopy; LCBI, lipid core burden index; IQR, interquartile range; IVUS, intravascular ultrasound.

Association between LCBI and MACE

The cumulative distribution of the $MaxLCBI_{4mm}$ values in patients with and without MACE shows that patients with MACE had higher $MaxLCBI_{4mm}$ values as compared to those without MACE (Figure 1).

Quartiles of $MaxLCBI_{4mm}$, $MaxLCBI_{10mm}$ and $LCBI_{ROI}$, and cumulative MACE incidence were pairwise compared. Pairwise comparisons consequently showed that patients in the third and fourth quartiles had significantly higher event rates compared to those in the first quartile (Figure 2). After adjustment for clinical characteristics and IVUS-derived plaque burden in the multivariable model, the third and fourth quartile of $MaxLCBI_{4mm}$ remained significantly associated with MACE (HR 3.09 (95%CI: 1.41-6.74) and HR 3.58 (95CI%: 1.67-7.70), respectively). Results for the $LCBI_{ROI}$ and $MaxLCBI_{10mm}$ were comparable (Table 2).

There was a statistically significant continuous relationship between higher $MaxLCBI_{4mm}$ values and a higher risk of MACE (Table 3). After multivariable adjustment, $MaxLCBI_{4mm}$ remained significantly associated with MACE (HR 1.19 per 100 units increase in LCBI, 95%CI: 1.07-1.32), as well as with MACE after exclusion of target lesion-related events (HR 1.21 (95CI%: 1.08-1.35)). Similarly, $MaxLCBI_{4mm}$ remained also independently associated with MACE after exclusion of adverse events related to the NIRS-imaged coronary segment (HR 1.19 (95%CI:1.06-1.34)). Cox regression analysis with follow-up duration as time-dependent variable demonstrated that continuous $MaxLCBI_{4mm}$ also predicted MACE beyond 1-year of follow-up [HR (95%CI) 1.15 (1.00-1.33) versus 1.23 (1.07-1.42) for the first year].

The C-indices indicate that NIRS-derived LCBI has prognostic value in addition to clinical risk factors and IVUS-derived plaque burden, with C-indices of the models with only covariates ranging from 0.607 to 0.617 and C-indices of the multivariable models including continuous LCBI ranging from 0.674 to 0.704 (Table 3).

Association between LCBI and the composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization

The cumulative incidence of the composite of cardiac death, non-fatal ACS or unplanned revascularization was higher in patients in the second (25.0%), third (31.3%) and fourth (35.7%) quartile of $MaxLCBI_{4mm}$ as compared to those in the first (10.3%) quartile of $MaxLCBI_{4mm}$ (log-rank pairwise comparisons p=0.031, p=0.002 and p=<0.001, respectively, Figure 3). The second, third and fourth quartiles of $MaxLCBI_{4mm}$ were significantly associated with the composite of cardiac death, non-fatal ACS or unplanned revascularization after adjustment for clinical characteristics and IVUS-derived plaque burden in the multivariable model (Table 4). A similar significant association was observed for $MaxLCBI_{4mm}$ as a continuous variable (Table 5). This association persisted after exclusion of target lesion-related events and after exclusion of events related to the imaged segment (Table

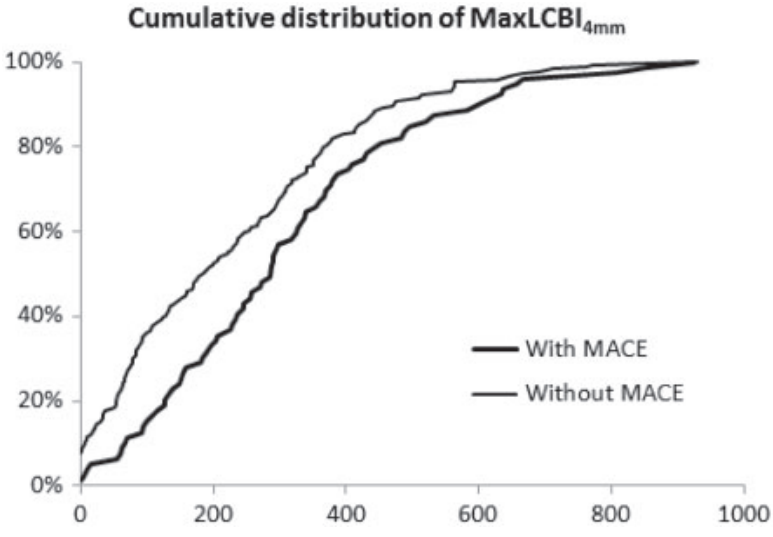

Figure 1. Cumulative distribution of the MaxLCBI$_{4mm}$ of patients with and without MACE (p=0.001, Mann-Whitney U test).
MACE: major adverse cardiac events, MaxLCBI$_{4mm}$: the 4 mm long segment with the maximum LCBI

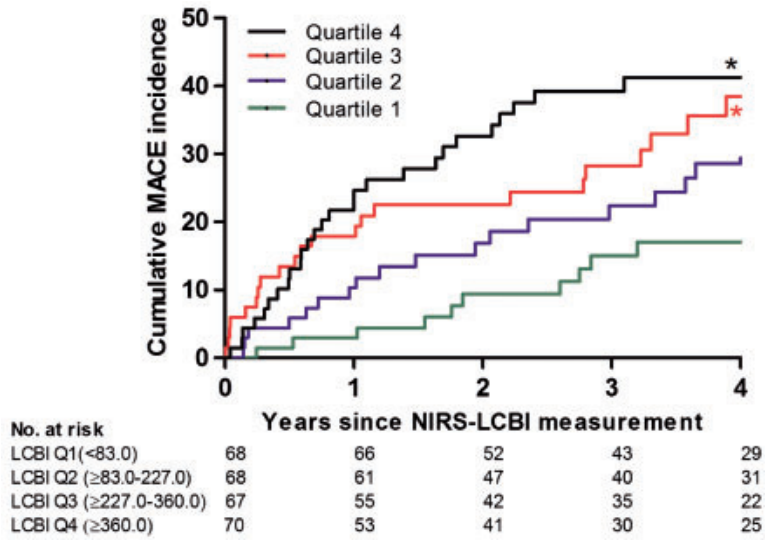

Figure 2. Assocation between quartiles of MaxLCBI$_{4mm}$ and the occurrence of MACE
*p<0.01 as compared to first quartile (reference).
LCBI: lipid core burden index, MACE: major adverse cardiac events

Table 2. Associations between quartiles of LCBI and risk of MACE at 4-years follow-up

	Cut-off LCBI value	Cumulative MACE incidence (%)	Unadjusted model		Multivariable model	
			HR (95%CI)	p-value	HR (95%CI)	p-value
$MaxLCBI_{4mm}$						
Quartile 1	0-83	14.7	1		1	
Quartile 2	≥83-227	27.9	1.99 (0.93-4.28)	0.078	2.11 (0.96-4.60)	0.062
Quartile 3	≥227-360	34.3	2.77 (1.32-5.81)	0.007	3.09 (1.41-6.74)	0.005
Quartile 4	≥360	38.6	3.22 (1.56-6.65)	0.002	3.58 (1.67-7.70)	0.001
$MaxLCBI_{10mm}$						
Quartile 1	0-48	13.2	1		1	
Quartile 2	≥48-129	30.9	2.56 (1.17-5.60)	0.018	2.66 (1.20-5.93)	0.017
Quartile 3	≥129-234	36.8	3.36 (1.57-7.20)	0.002	3.47 (1.59-7.61)	0.002
Quartile 4	≥234	34.3	3.06 (1.42-6.59)	0.004	3.27 (1.46-7.29)	0.004
$LCBI_{ROI}$						
Quartile 1	0-13	15.4	1		1	
Quartile 2	≥13-40	25.7	1.72 (0.79-3.73)	0.17	1.93 (0.88-4.25)	0.10
Quartile 3	≥40-79	37.1	2.90 (1.40-6.02)	0.004	3.24 (1.53-6.88)	0.002
Quartile 4	≥79	35.7	2.67 (1.28-5.56)	0.009	3.14 (1.43-6.87)	0.004

Cumulative MACE incidence by Kaplan-Meier method. P-values obtained with Cox regression analyses on pairwise comparisons between each quartile and first quartile (reference).
HR: hazard ratio, LCBI, lipid core burden index; MACE, major adverse cardiac events, ROI: region of interest

Table 3. Continuous LCBI values and risk of MACE at 4-years follow-up

	Unadjusted model		Multivariable model		C-index
	HR(95%CI)	p-value	HR(95%CI)	p-value	
MACE					
Covariates only					0.608
$MaxLBCI_{4mm}$	1.19 (1.08-1.31)	0.001	1.19 (1.07-1.32)	0.001	0.674
$MaxLBCI_{10mm}$	1.17 (1.04-1.31)	0.011	1.17 (1.03-1.34)	0.017	0.660
$LCBI_{ROI}$	1.18 (0.93-1.51)	0.18	1.24 (0.95-1.63)	0.12	0.652
MACE with exclusion of TLR-events					
Covariates only					0.617
$MaxLBCI_{4mm}$	1.22 (1.10-1.36)	<0.001	1.21 (1.08-1.35)	0.001	0.704
$MaxLBCI_{10mm}$	1.21 (1.07-1.37)	0.003	1.22 (1.06-1.40)	0.005	0.691
$LCBI_{ROI}$	1.24 (0.97-1.60)	0.087	1.31 (0.99-1.74)	0.059	0.683
MACE with exclusion of NIRS imaged segment-related events					
Covariates only					0.607
$MaxLBCI_{4mm}$	1.17 (1.06-1.30)	0.003	1.19 (1.06-1.34)	0.003	0.683
$MaxLBCI_{10mm}$	1.13 (0.99-1.28)	0.072	1.15 (1.00-1.33)	0.050	0.665
$LCBI_{ROI}$	1.09 (0.81-1.46)	0.58	1.18 (0.86-1.62)	0.31	0.659

Hazard ratios per 100 units increase in $MaxLCBI_{4mm}$, $MaxLCBI_{10mm}$ and $LCBI_{ROI}$.
HR: hazard ratio, LCBI: lipid core burden index, MACE: major adverse cardiac events, NIRS: near-infrared spectroscopy, ROI: region of interest, TLR: target lesion-related revascularization

Table 4. Associations between quartiles of LCBI and risk of composite of cardiac death, non-fatal ACS, or unplanned revascularization at 4-years follow-up

	Cut-off LCBI value	Cumulative incidence (%)	Unadjusted model		Multivariable model	
			HR(95%CI)	p-value	HR(95%CI)	p-value
$MaxLCBI_{4mm}$						
Quartile 1	0-83	10.3	1		1	
Quartile 2	≥83-227	25.0	2.53 (1.05-6.11)	0.039	2.66 (1.09-6.50)	0.032
Quartile 3	≥227-360	31.3	3.60 (1.53-8.46)	0.003	4.07 (1.67-9.92)	0.002
Quartile 4	≥360	35.7	4.16 (1.80-9.62)	0.001	4.57 (1.90-10.98)	0.001
$MaxLCBI_{10mm}$						
Quartile 1	0-48	10.3	1		1	
Quartile 2	≥48-129	26.5	2.80 (1.17-6.70)	0.021	2.96 (1.21-7.21)	0.017
Quartile 3	≥129-234	30.9	3.60 (1.53-8.47)	0.003	3.73 (1.55-8.94)	0.003
Quartile 4	≥234	34.3	3.85 (1.66-8.93)	0.002	4.01 (1.66-9.67)	0.002
$LCBI_{ROI}$						
Quartile 1	0-13	10.8	1		1	
Quartile 2	≥13-40	21.4	2.05 (0.84-5.02)	0.12	2.30 (0.93-5.73)	0.073
Quartile 3	≥40-79	32.9	3.64 (1.56-8.49)	0.003	4.09 (1.72-9.73)	0.001
Quartile 4	≥79	35.7	3.73 (1.61-8.62)	0.002	4.18 (1.72-10.17)	0.002

Cumulative endpoint incidence by Kaplan-Meier method. P-values obtained with Cox regression analyses on pairwise comparisons between each quartile and first quartile (reference).
ACS: acute coronary syndrome, CI: confidence interval, HR: hazard ratio, LCBI: lipid core burden index, ROI: region of interest

Table 5. Continuous LCBI values and risk of composite of cardiac death, non-fatal ACS or unplanned revascularization at 4-years follow-up

	Unadjusted model		Multivariable model	
	HR(95%CI)	p-value	HR(95%CI)	p-value
$MaxLBCI_{4mm}$	1.21 (1.10-1.34)	<0.001	1.21 (1.08-1.35)	0.001
$MaxLBCI_{10mm}$	1.20 (1.06-1.35)	0.003	1.20 (1.05-1.37)	0.007
$LCBI_{ROI}$	1.24 (0.98-1.58)	0.078	1.29 (0.98-1.70)	0.065
Composite endpoint with exclusion of TLR-events				
$MaxLBCI_{4mm}$	1.25 (1.12-1.40)	<0.001	1.24 (1.10-1.39)	<0.001
$MaxLBCI_{10mm}$	1.25 (1.10-1.42)	0.001	1.25 (1.09-1.44)	0.002
$LCBI_{ROI}$	1.32 (1.03-1.68)	0.027	1.38 (1.04-1.83)	0.027
Composite endpoint with exclusion of NIRS imaged segment-related events				
$MaxLBCI_{4mm}$	1.20 (1.08-1.34)	0.001	1.22 (1.08-1.38)	0.001
$MaxLBCI_{10mm}$	1.16 (1.02-1.33)	0.026	1.19 (1.03-1.38)	0.022
$LCBI_{ROI}$	1.16 (0.87-1.55)	0.31	1.24 (0.90-1.70)	0.18

Hazard ratios per 100 units increase in $MaxLCBI_{4mm}$, $MaxLCBI_{10mm}$ and $LCBI_{ROI}$.
ACS: acute coronary syndrome, LCBI: lipid core burden index, NIRS: near-infrared spectroscopy, ROI: region of interest, TLR: target lesion-related revascularization

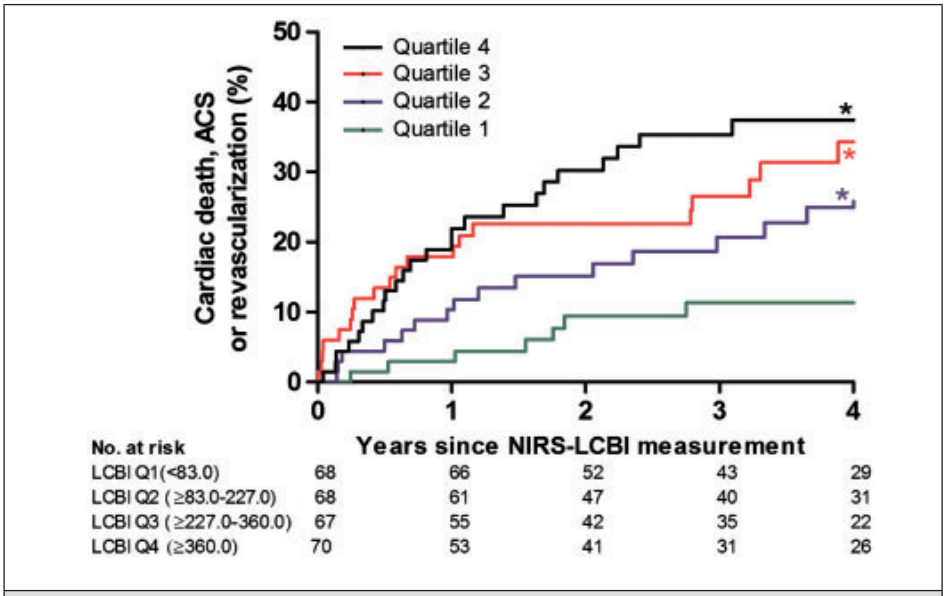

Figure 3. Assocation between quartiles of MaxLCBI$_{4mm}$ and the occurrence of *cardiac* death, non-fatal ACS and unplanned revascularization

*p<0.05 as compared to first quartile (reference).

LCBI: lipid core burden index, MACE: major adverse cardiac events, NIRS: near-infrared spectroscopy

5). In general, the associations observed between MaxLCBI$_{4mm}$, MaxLCBI$_{10mm}$ and LCBI$_{ROI}$ and the occurrence of adverse events were stronger and more significant when the composite endpoint included cardiac death instead of all-cause mortality (Tables 2-5).

DISCUSSION

This study investigated the association between lipid rich core-containing plaques as identified by NIRS in a non-culprit coronary artery and the occurrence of adverse cardiac events during long-term follow-up in patients undergoing CAG. This study showed that LCBI values were significantly and independently associated with the incidence of adverse cardiac outcome in patients with CAD over 4 years of follow-up. To the best of our knowledge, this is the first study to investigate the association between LCBI in a non-culprit coronary artery and adverse cardiac outcome over 4 years of follow-up, which represents the longest follow-up period so far reported.

Studies on the relationship between LCBI and (long-term) follow-up are scarce. Recently, the COLOR study demonstrated that the MaxLCBI$_{4mm}$ obtained prior to stenting in a culprit coronary segment was not associated with culprit-related MACE during 2 years of follow-up.[15] Our study provides new evidence on the prognostic value of NIRS, since

we demonstrated that NIRS is predictive of MACE on the long-term by identifying high-risk lipid rich core-containing plaques in a non-culprit artery. The upcoming Lipid Rich Plaque (LRP) and PROSPECT-2 studies are also investigating the ability of NIRS-derived LCBI in non-culprit coronary arteries to predict adverse cardiovascular outcome during 2-year follow-up.

This study extends our previous 1-year follow-up data of the ATHEROREMO-NIRS study, which investigated the 1-year prognostic value of NIRS in that cohort and showed that high LCBI values were associated with an increased incidence of MACE.[10] The current study demonstrated that these results persist over a period of 4 years, suggesting that the increased risk at 1-year was not due to chance and LCBI of a non-culprit artery also has prognostic value beyond 1-year after the index CAG. As compared to the 1-year follow-up, the current study was conducted over a longer follow-up period, had a larger sample size and, consequently, a larger number of endpoints. The latter allowed us to investigate the associations between continuous LCBI values, as well as quartiles of LCBI, and adverse cardiac outcome instead of using a median split for LCBI. These analyses showed a significant and independent continuous relationship between higher LCBI values in a non-culprit coronary artery and adverse cardiac outcome. Importantly, this relationship persisted, and remained essentially unchanged, when target-lesion related adverse cardiac events (TLR) were excluded from the study endpoint, as well as when adverse events related to the imaged coronary segment were excluded. This indicates that LCBI values obtained in a non-culprit coronary artery segment are associated with adverse cardiac events throughout the entire coronary tree. As such, this finding supports the hypothesis that NIRS imaging in a non-culprit coronary artery segment may reflect vulnerability of the entire coronary tree.[8,16]

Previously, the ATHEROREMO-IVUS study demonstrated that IVUS-derived imaging parameters were predictive of MACE. For this reason, we included IVUS-derived plaque burden in the multivariable model to evaluate the independent prognostic value of NIRS. Given that progression of coronary atherosclerosis depends on multiple factors that are cumulative, interactive and nonlinear, a combination of these two imaging techniques is likely to result in a higher predictive value.

Other studies used NIRS to investigate the effect of anti-atherosclerotic therapy on the amount of lipid core-containing plaques. The YELLOW study demonstrated that patients with multivessel CAD treated for 6 to 8 weeks with rosuvastatin showed a reduction of lipid core in obstructive arteries.[17] The IBIS-3 study showed that high-dose rosuvastatin resulted in a neutral effect on lipid rich core-containing plaques as determined by NIRS.[18] Recently, it was shown that addition of a PCSK9-inhibitor to stable statin therapy resulted in a greater decrease of plaque burden as assessed by IVUS.[19] NIRS has improved ability to identify lipid core-containing coronary plaques as compared to other invasive imaging modalities including IVUS, since NIRS is able to distinguish cholesterol from other

tissue characteristics.[6] In this context, NIRS may be used to select patients with high LCBI values in future research to measure the effect of anti-atherosclerotic therapy on lipid rich core-containing plaques in the coronary artery wall and assess its association with adverse cardiac outcome. Ultimately, this may result in improved risk stratification and management of patients with CAD.

Limitations

Several study limitations warrant consideration. First, our study population also comprised patients from IBIS-3, who received high doses of rosuvastatin after the index procedure. This may also in part have affected the effect estimates. However, a post-hoc analysis did not display significant effect modification according to study.

Second, the follow-up questionnaire was completed by 90% of the patients. Although for the majority of the remaining patients, follow-up information was retrieved from our local hospital records, we cannot fully exclude the possibility that loss to follow-up was in part selective. However, a post-hoc analysis of clinical and NIRS characteristics of the non-responders as compared to those of the responders did not show any differences that indicated selective loss to follow-up.

Third, the sample size of this single-center study was relatively small. Nevertheless, our study had a large number of endpoints. This allowed us to analyse LCBI as quartiles and as a continuous variable, as well as to investigate the association with adverse cardiac outcome after exclusion of target lesion-related and imaged segment-related events. When the results of the LRP and PROSPECT-2 studies become available, a meta-analysis may provide more precise effect estimates. Furthermore, as the current study population comprises a broad spectrum of CAD patients, the results are expected to apply to a broad population of CAD patients.

Conclusions

In conclusion, this study demonstrates for the first time that LCBI, as assessed by NIRS in one non-culprit coronary artery segment, predicts adverse cardiac outcome, independent of clinical characteristics and IVUS, during long-term follow-up over 4 years in patients referred for CAG because of ACS or SAP.

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CORONARY VULNERABILITY

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11

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.

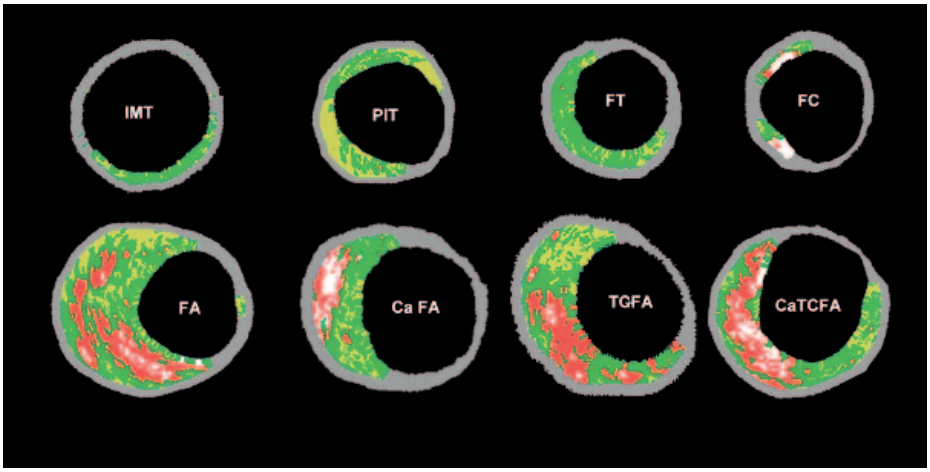


Figure 1. Classification of plaque morphology with intravascular ultrasound virtual histology
IMT, intimal medial thickening; PIT, pathological intimal thickening; FT, fibrotic plaque; FC, fibrocalcific plaque; FA, fibroatheroma; CaFA, calcified fibroatheroma; TCFA, thin-cap fibroatheroma; CaTCFA, calcified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

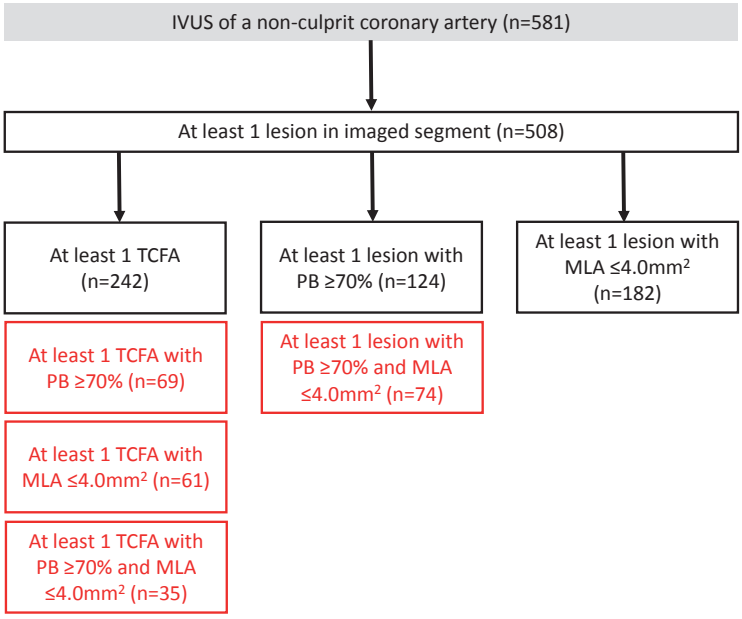


Figure 2. Study participants and presence of high risk coronary lesions on intravascular ultrasound
IVUS, intravascular ultrasound; MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma.

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

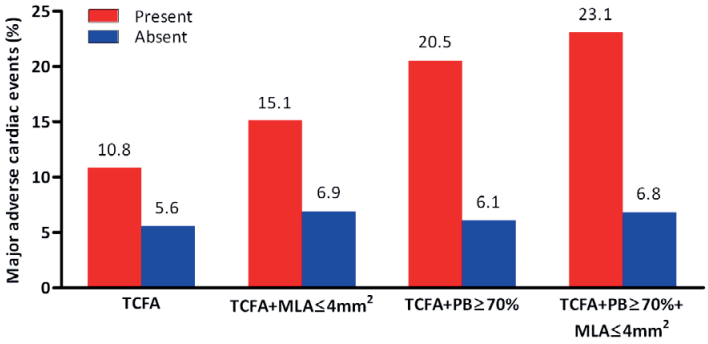
Patients who did not had any lesion in the imaged coronary segment seemed to have lower occurrence of MACE (absent 4.1% vs. present 8.3%; HR 0.48, 95% CI 0.15-1.54; p=0.22) and lower occurrence of the composite of death or ACS only (absent 1.4% vs. present 5.4%; HR 0.25, 95% CI 0.034-1.83; p=0.17), although 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

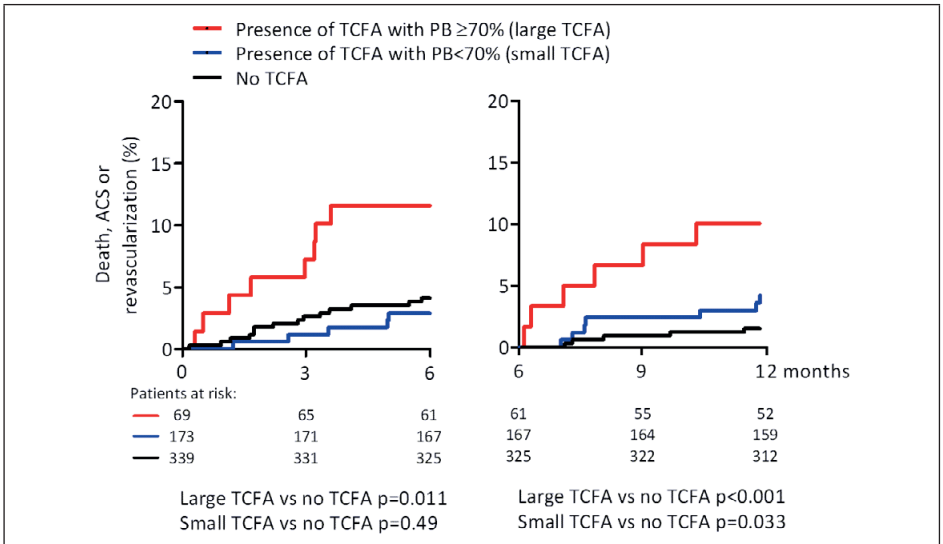


Figure 4. Associations with of short-term and long-term major adverse cardiac events
P values are obtained with log-rank test. Overall P value 0-6 months is 0.009; overall P value 6-12 months is 0.002.
PB, plaque burden; TCFA, thin-cap fibroatheroma.

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.

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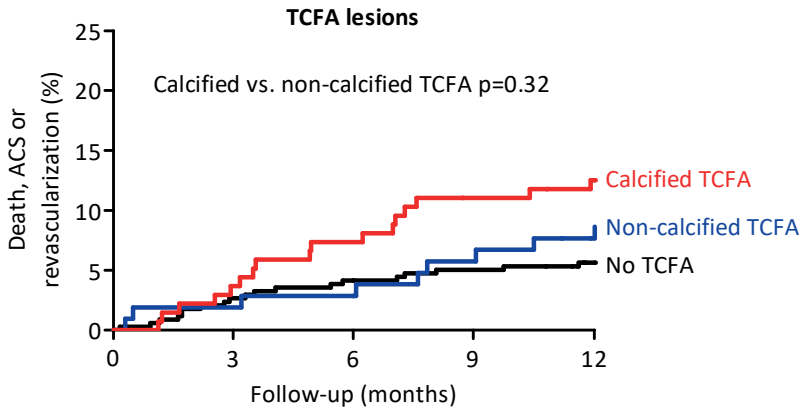
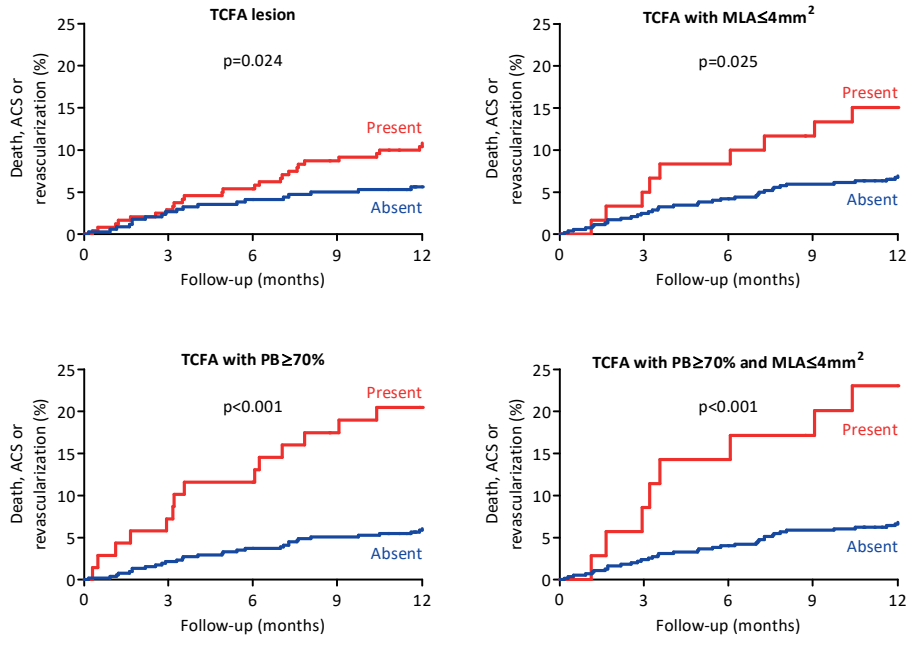
SUPPLEMENTAL FIGURES AND TABLES



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.

CORONARY VULNERABILITY

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PROGNOSTIC VALUE OF INTRAVASCULAR ULTRASOUND IN PATIENTS WITH CORONARY ARTERY DISEASE

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ABSTRACT

Background: Intravascular ultrasound (IVUS) and radiofrequency (RF-)IVUS have shown to be able to detect high-risk coronary plaque characteristics.

Objectives: We studied the long-term prognostic value of (RF-)IVUS-derived plaque characteristics in patients with coronary artery disease (CAD) undergoing coronary angiography.

Methods: During 2008-2011, (RF-)IVUS was performed in one non-stenotic segment of a non-culprit coronary artery in 581 patients undergoing coronary angiography for acute coronary syndrome (ACS) or stable angina. The predefined primary endpoint was MACE, defined as the composite of all-cause death, non-fatal ACS or unplanned revascularization. Hazard ratios (HR) were adjusted for age, sex and clinical risk factors.

Results: During a median follow-up of 4.7 years, 152 patients (26.2%) had MACE. The presence of a lesion with a minimal luminal area ≤4.0mm^2 was independently associated with MACE (HR:1.49, 95%CI:1.07-2.08, p=0.020), whereas the presence of a thin-cap fibroatheroma lesion or a lesion with a plaque burden ≥70% on their own were not. Results were comparable when the composite endpoint included cardiac death instead of all-cause death. The presence of a lesion with a plaque burden of ≥70% was independently associated with the composite endpoint of cardiac death, nonfatal ACS or unplanned revascularization after exclusion of culprit-lesion related events (HR:1.66, 95%CI:1.06-2.58, p=0.026). Likewise, each 10 units increase in segmental plaque burden was independently associated with a 26% increase in risk of this composite endpoint (HR:1.26 per 10 units increase, 95%CI:1.03-1.52, p=0.022).

Conclusions: IVUS-derived small luminal area and large plaque burden, and not RF-IVUS-derived compositional plaque features on their own, predict adverse cardiovascular outcome during long-term follow-up in patients with CAD.

INTRODUCTION

Patients with coronary artery disease (CAD) are at increased risk of recurrent adverse cardiovascular events, such as acute coronary syndromes (ACS).(1,2) Whereas coronary angiography (CAG) only yields a two-dimensional silhouette of the lumen,(3) greyscale intravascular ultrasound (IVUS) and radiofrequency (RF-)IVUS have shown to be able to identify high-risk coronary plaque characteristics within the coronary artery wall. (4-7) Therefore, (RF-)IVUS may be useful to identify patients at increased risk of future adverse cardiovascular events.(6-8) Autopsy studies suggest that an ACS is often caused by rupture or fissure of a thin-cap fibroatheroma (TCFA), a vulnerable coronary plaque containing a large lipid-rich necrotic core overlaid by a thin inflamed fibrous cap.(9-12) Identification of this vulnerable coronary plaque phenotype by invasive imaging may therefore improve risk stratification and management of CAD patients.

To date, a few studies have investigated the prognostic value of (RF-)IVUS for adverse cardiovascular outcome.(13,14) The PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) study demonstrated that (RF-)IVUS-derived high-risk plaque characteristics in the three major coronary arteries predict adverse cardiac events in patients admitted with ACS during long-term follow-up.(13) However, patients with stable angina pectoris (SAP) were not included in PROSPECT and the number of endpoint events in that study was primarily driven by rehospitalizations. Our ATHEROREMO-IVUS (European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound) study demonstrated that high-risk plaque characteristics, as derived by (RF-)IVUS in one non-stenotic segment of a non-culprit coronary artery were predictive of adverse cardiovascular events in a broad spectrum of patients with CAD, including SAP, at 1-year follow-up.(15) We now report the long-term (median 4.7 years) follow-up data.

METHODS

Study design and population

The design of the ATHEROREMO-IVUS study has been described in detail elsewhere. (15,16) Briefly, between 2008 and 2011, 581 patients undergoing diagnostic CAG or percutaneous coronary intervention (PCI) for ACS or SAP underwent (RF-)IVUS imaging of a non-culprit coronary artery in the Erasmus MC, Rotterdam, The Netherlands.(15,16) Baseline (RF-)IVUS images were analyzed off-line and were not used for patient care. Thereafter, patients were followed-up on adverse cardiovascular outcome.

The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC and was performed in accordance with the declaration of Helsinki. All

patients provided written informed consent which included approval for long-term follow-up. The ATHEROREMO-IVUS study was registered in ClinicalTrialsgov.org, number NCT01789411.

Intravascular ultrasound

Subsequent to the standard index CAG, (RF-)IVUS imaging was performed in a non-stenotic segment of a non-culprit coronary artery. The target segment in this non-culprit coronary artery was required to be at least 40 mm in length and without significant luminal narrowing (<50% stenosis) as assessed by on-line angiography. The order of preference for selection of the non-culprit vessel was; (i) left anterior descending artery, (ii) right coronary artery, (iii) left circumflex artery.(15,16) IVUS images were acquired by the Volcano s5/s5i Imaging system, including a Volcano Eagle Eye Gold IVUS catheter (20 MHz) that was automatically pulled back at a standard speed of 0.5 mm/s (Volcano Corp., San Diego, CA, USA). Greyscale- and RF-IVUS data were analyzed off-line by an independent core laboratory (Cardialysis, Rotterdam, The Netherlands) using the pcVH 2.1 and qVH software (Volcano Corp., San Diego, CA, USA). The core laboratory was blinded to all other patient characteristics and outcome data.

Greyscale IVUS measurements included segmental plaque volume and plaque burden. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Segmental plaque burden was defined as the plaque and media cross-sectional area divided by the 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 RF-IVUS analyses, compositional features of coronary lesions were classified as fibrous, fibro-fatty, necrotic core or dense calcium.(5,15,16) Confluent necrotic core or dense calcium, or the contact of necrotic core with the lumen, were assessed by visual examination performed independently by three investigators blinded to outcome data. Coronary lesions were further classified into 8 different lesion types.(7,15,16) The mentioned criteria should be present in three consecutive frames for a lesion to be considered of a particular category. Three lesions, as identified by (RF-)IVUS, were considered as lesions associated with a high risk for subsequent adverse cardiac events; 1) TCFA lesion, defined as a lesion with the presence of >10% confluent necrotic core in direct contact with the lumen; 2) lesion with a plaque burden ≥70%; 3) lesion with a minimal luminal area ≤4.0mm^2.(15)

Follow-up

Follow-up was reported by January 2015. Vital status of the patients was obtained from municipal civil registries. Subsequently, as a first screening method, follow-up questionnaires were sent to all living patients for identifying possible adverse events. Thereafter, hospital discharge letters were obtained if any hospitalization or possible event was re-

ported. In patients who did not return the questionnaire, the local hospital records were investigated for possible events. Cause of death was obtained from hospital records, autopsy reports or general practitioner notes.

Study endpoints

The predefined primary endpoint consisted of major adverse cardiovascular events (MACE), defined as the composite of all-cause death, non-fatal ACS, or unplanned revascularization during long-term follow-up. In accordance with our previous studies on the prognostic value of (RF-)IVUS and near-infrared spectroscopy (NIRS) in this study population, we also performed a predefined analysis on the composite endpoint of *cardiac* death, non-fatal ACS, or unplanned revascularization. This analysis was performed based on the pathophysiological concept that (RF-)IVUS-derived plaque characteristics would hypothetically be more likely associated with (atherosclerotic-driven) cardiovascular events and not with definite non-cardiac events (such as death because of malignancy). Similarly, an additional analysis was performed on this endpoint after exclusion of *definite* culprit lesion-related events. This exploratory analysis aimed to assess the question as to whether the atherosclerotic burden, as assessed in a single, non-culprit coronary artery segment, would reflect vulnerability of the entire coronary tree.

In accordance with the guidelines of the European Society of Cardiology, non-fatal ACS was defined as the clinical diagnosis of ST-segment Elevation Myocardial Infarction (STEMI), non-STEMI, or unstable angina.(17,18) Unplanned coronary revascularization was defined as urgent revascularization for ACS or unplanned (i.e. not part of pre-planned multi-stage PCI) elective revascularization for progressive angina pectoris. Cardiac death was defined as any death due to proximate cardiac cause, unwitnessed death or death of unknown cause.

Based on original source data of available coronary angiography and hospital records at the time of the event, the clinical event committee adjudicated (blinded to IVUS data) whether the event was related to the coronary site that had been treated during the index procedure (culprit lesion-related event) or as related to a coronary site that had not been 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.(15)

Statistical analysis

Normally-distributed continuous variables were reported as means and standard deviations. Non-normally-distributed variables were reported as medians and interquartile ranges (IQR). Categorical variables were reported as numbers and percentages.

Cumulative events rates were estimated by the Kaplan-Meier method and differences between groups were evaluated by the Log-rank test. Patients that were lost to follow-up were censored at the date of last contact. In case a patient had multiple events, the first event was counted for the composite endpoint.

The associations between (RF-)IVUS characteristics and study endpoints were further analyzed by Cox proportional hazard regression analysis. We applied multivariable Cox regression, with adjustment for age, sex, diabetes mellitus, hypertension, dyslipidemia, indication for CAG (ACS or SAP), history of myocardial infarction, history of PCI, history of CABG, history of peripheral artery disease and PCI performed at index procedure. These potential confounders were chosen based on clinical relevance or their significant association with MACE in univariable Cox regression analysis. Hazard ratios (HRs) were reported with 95% confidence intervals (95% CIs).

In case the composite endpoint was defined with exclusion of culprit lesion-related events, the occurrence of a culprit lesion-related event as a first event during follow-up was not counted and the patient was not censored as this patient is considered to be still at risk of a non-culprit lesion-related or indeterminate event during further follow-up. When the composite endpoint was based on non-culprit lesion-related or indeterminate events, patients were only censored in case a non-culprit lesion-related or indeterminate event occurred, if they were lost-to-follow-up or if they died.

All statistical tests were two-tailed and p-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS statistics version 21.0 (IBM Corp., Armonk, New York).

RESULTS

Baseline characteristics

Mean age of the patients was 61.6 ± 11.3 years, 75.6% were men and 54.7% presented with an ACS (Table 1). Median segmental plaque burden was 39.1 (IQR: 30.0-46.4)%, and plaque volume was 222.7 (IQR: 136.1-326.6)mm^3. On the basis of (RF-)IVUS, 724 lesions were identified in 508 (87.4%) patients that had at least one lesion in the imaged segment, including 127 (17.5%) lesions with a plaque burden ≥70% in 124 (21.3%) patients, 206 (28.5%) lesions with a minimal luminal area ≤4.0 mm^2 in 182 (31.3%) patients and 74 (10.2%) lesions with both plaque characteristics in 74 (12.7%) patients. On the basis of RF-IVUS, 271 (37.4%) TCFA lesions were identified in 242 (41.7%) patients, including 71 (9.8%) TCFA lesions with a plaque burden ≥70% in 69 patients (11.9%), 61 (8.4%) TCFA lesions with a minimal luminal area ≤4.0 mm^2 in 61 (10.5%) patients and 35 (4.8%) TCFA lesions with both plaque characteristics in 35 (6.0%) patients.

Table 1. Baseline characteristics

	N = 581 patients
Clinical characteristics	
Age, years	61.6 ± 11.3
Men, n (%)	439 (75.6)
Diabetes Mellitus, n (%)	99 (17.0)
Hypertension, n (%)	300 (51.6)
Dyslipidemia, n (%)	321 (55.2)
Current 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 CVA, n (%)	26 (4.5)
History of peripheral artery disease, n (%)	36 (6.2)
History of renal impairment, n (%)	32 (5.5)
History of heart failure, n (%)	19 (3.3)
Median C-reactive protein, mg/L	2.1 [0.9-5.4]
Procedural characteristics	
Indication for coronary angiography	
Acute MI, n (%)	167 (28.7)
Unstable angina, n (%)	151 (26.0)
Stable angina, n (%)	254 (43.7)
Other, n (%)	9 (1.5)
PCI performed, n (%)	511 (88.0)
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)
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)
Median imaged segment length, mm	44.3 [33.8-55.4]
Median segmental plaque burden, %	39.1 [30.0-46.4]
Median segmental plaque volume, mm^3	222.7 [136.1-326.6]

CABG, coronary artery bypass graft; CVA, cerebrovascular accident; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Incidence of study endpoints

Median follow-up time was 4.7 (IQR: 4.2-5.6) years. Follow-up questionnaires were sent to all 528 (90.9%) living patients and were completed by 86%. The predefined composite endpoint of all-cause death, non-fatal ACS or unplanned revascularization occurred in 152 patients (26.2%) (Table 2). A total of 27 events were classified as definite culprit lesion-related, 72 as non-culprit lesion-related and 53 as indeterminate event (Table 2). The composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization occurred in 125 patients (21.5%) (Table 2). The composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization after exclusion of definite culprit lesion-related events occurred in 98 patients (16.9%) (Table 2).

Table 2. Incidence of composite endpoints.

	Definite CLR events	Definite non-CLR events	Indeterminate events	Non-CLR and indeterminate events combined	All events
Composite of MACE, n	27	72	53	125	152[a]
Death from any cause, n	1	11	38	49	50
Cardiac death, n	1	4	20	24	25
Nonfatal ACS, n	13	24	10	34	47
Unplanned revascularization, n	13	37	5	42	55
Composite of cardiac death, nonfatal ACS or unplanned revascularization, n	27	63	35	98[c]	125[b]

a. Composite of MACE; all-cause death, nonfatal ACS or unplanned revascularization.
b. Composite of cardiac death, nonfatal ACS or unplanned revascularization.
c. Non-culprit lesion-related and indeterminate cardiac death, nonfatal ACS or unplanned revascularization.
ACS, acute coronary syndrome; CLR, culprit lesion-related; MACE, major adverse cardiovasulcar events.
Numbers refer to the first event counted for the composite endpoint.

Association between (RF-)IVUS and MACE

The presence of a lesion with a minimal luminal area ≤4.0 mm^2 was significantly and independently associated with MACE (cumulative MACE incidence when present: 33.9% vs. 22.2% when absent; adjusted HR: 1.49, 95% CI: 1.07-2.08, p=0.020) (Table 3). Furthermore, the presence of a TCFA lesion with a plaque burden ≥70% was significantly associated with MACE (cumulative MACE incidence when present: 37.7% vs. 24.6% when absent; adjusted HR: 1.73, 95% CI: 1.12-2.66, p=0.013), while the presence of a TCFA lesion or a lesion with a plaque burden ≥70% itself was not independently associated with MACE (Table 3). After multivariable adjustment, segmental plaque burden and plaque volume remained no longer independently associated with MACE (Table 3). Re-

sults were essentially similar when definite culprit lesion-related events were excluded. Cox regression analysis with follow-up duration as time-dependent variable showed that both the presence of a TCFA lesion and a lesion with a plaque burden ≥70% were strong predictors of MACE for the first year of follow-up, but not beyond 1-year follow-up. On the contrary, a lesion with a minimal luminal area ≤4.0 mm^2 itself was not an independent predictor in the first year of follow-up (adjusted HR: 1.40, 95% CI: 0.83-2.34, p=0.21), but did predict MACE beyond 1-year of follow-up (1-year to 5-year follow-up adjusted HR: 1.58, 95% CI: 1.04-2.40, p=0.032). Results remained essentially similar when we performed an exploratory multivariable analysis applying the model used for the 1-year follow-up data (which comprised 6 variables instead of the 11 variables used in the model for the current analyses) (Supplemental table 1).

Table 3. Associations of (RF-)IVUS and risk of adverse cardiac events at 4.7-years follow-up

	Unadjusted model HR (95% CI)	P-value	Full model HR (95% CI)	P-value
MACE				
TCFA	1.20 (0.87-1.65)	0.27	1.27 (0.91-1.77)	0.16
PB ≥70%	1.50 (1.05-2.16)	0.028	1.33 (0.92-1.93)	0.13
MLA ≤4.0 mm^2	1.57 (1.13-2.17)	0.007	1.49 (1.07-2.08)	0.020
TCFA + PB ≥70%	1.90 (1.25-2.90)	0.003	1.73 (1.12-2.66)	0.013
TCFA + MLA ≤4.0 mm^2	1.47 (0.93-2.33)	0.10	1.50 (0.93-2.44)	0.10
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.64 (0.93-2.89)	0.089	1.74 (0.97-3.13)	0.066
PB≥70% + MLA ≤4.0 mm^2	1.29 (0.83-2.01)	0.26	1.30 (0.82-2.04)	0.26
Segmental plaque burden, %	1.24 (1.07-1.44)	0.004	1.15 (0.98-1.34)	0.079
Segmental plaque volume, mm^3	1.07 (0.96-1.20)	0.23	1.02 (0.90-1.14)	0.79
Composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization				
TCFA	1.04 (0.73-1.49)	0.83	1.12 (0.77-1.61)	0.56
PB ≥70%	1.63 (1.10-2.42)	0.014	1.43 (0.96-2.15)	0.083
MLA ≤4.0 mm^2	1.85 (1.30-2.64)	0.001	1.82 (1.26-2.64)	0.001
TCFA + PB ≥70%	1.95 (1.23-3.09)	0.005	1.78 (1.11-2.85)	0.017
TCFA + MLA ≤4.0 mm^2	1.74 (1.08-2.81)	0.023	1.86 (1.11-3.10)	0.018
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.84 (1.02-3.34)	0.044	2.09 (1.12-3.89)	0.020
PB≥70% + MLA ≤4.0 mm^2	1.45 (0.91-2.32)	0.12	1.51 (0.93-2.45)	0.093
Segmental plaque burden, %	1.28 (1.09-1.50)	0.003	1.17 (0.99-1.39)	0.070
Segmental plaque volume, mm^3	1.06 (0.93-1.20)	0.40	0.98 (0.86-1.12)	0.80

Table 3 (*continued*)

	Unadjusted model HR (95% CI)	P-value	Full model HR (95% CI)	P-value
Composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization exclusive of culprit lesion-related events				
TCFA	0.99 (0.66-1.48)	0.95	1.07 (0.70-1.62)	0.76
PB ≥70%	2.08 (1.36-3.18)	0.001	1.66 (1.06-2.58)	0.026
MLA ≤4.0 mm^2	2.03 (1.37-3.03)	<0.001	1.88 (1.24-2.83)	0.003
TCFA + PB ≥70%	2.17 (1.32-3.59)	0.002	1.84 (1.10-3.07)	0.021
TCFA + MLA ≤4.0 mm^2	1.64 (0.95-2.84)	0.078	1.75 (0.98-3.13)	0.059
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.97 (1.02-3.79)	0.042	2.03 (1.03-4.02)	0.041
PB≥70% + MLA ≤4.0 mm^2	1.77 (1.07-2.92)	0.026	1.73 (1.04-2.90)	0.035
Segmental plaque burden, %	1.41 (1.18-1.69)	<0.001	1.26 (1.03-1.52)	0.022
Segmental plaque volume, mm^3	1.13 (0.99-1.30)	0.080	1.03 (0.89-1.20)	0.68

Hazard ratios per 10 and 100 units increase in segmental plaque burden and plaque volume, respectively. CI, confidence interval; HR, hazard ratio; IVUS, intravascular ultrasound; MACE, major adverse cardiovascular events; MLA, minimal luminal area; PB, plaque burden; RF, radiofrequency; TCFA, thin-cap fibroatheroma.

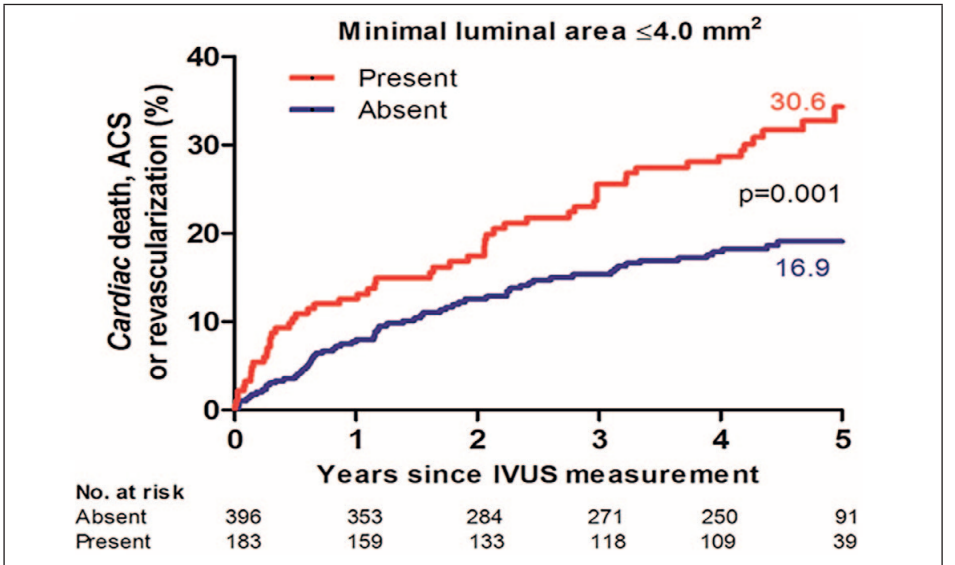

Figure 1. Association between the absence or presence of a lesion with a minimal luminal area ≤4.0 mm^2 and the composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization. P-value obtained by the Log-rank test.
ACS, acute coronary syndrome; IVUS, intravascular ultrasound

Association between (RF-)IVUS and the composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization

The presence of a lesion with a minimal luminal area ≤4.0 mm^2 was also significantly and independently associated with a higher rate of the composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization (cumulative incidence of composite endpoint when present: 30.6% vs. 16.9% when absent; adjusted HR: 1.82, 95% CI:1.26-2.64, p=0.001) (Figure 1 and Table 3). The same was true for TCFA lesions with a plaque burden ≥70% or a minimal luminal area ≤4.0 mm^2 (Table 3). The highest risk, in terms of adjusted HRs, was among patients who had a TCFA lesion with both a plaque burden ≥70% and a minimal luminal area ≤4.0 mm^2 (cumulative incidence of composite endpoint when present: 34.3% vs. 20.7% when absent; adjusted HR: 2.09, 95% CI: 1.12-3.89, p=0.020) (Table 3).

These associations remained essentially unchanged after *exclusion* of culprit lesion-related events (Figure 2 and Table 3). In addition, a significant association was observed

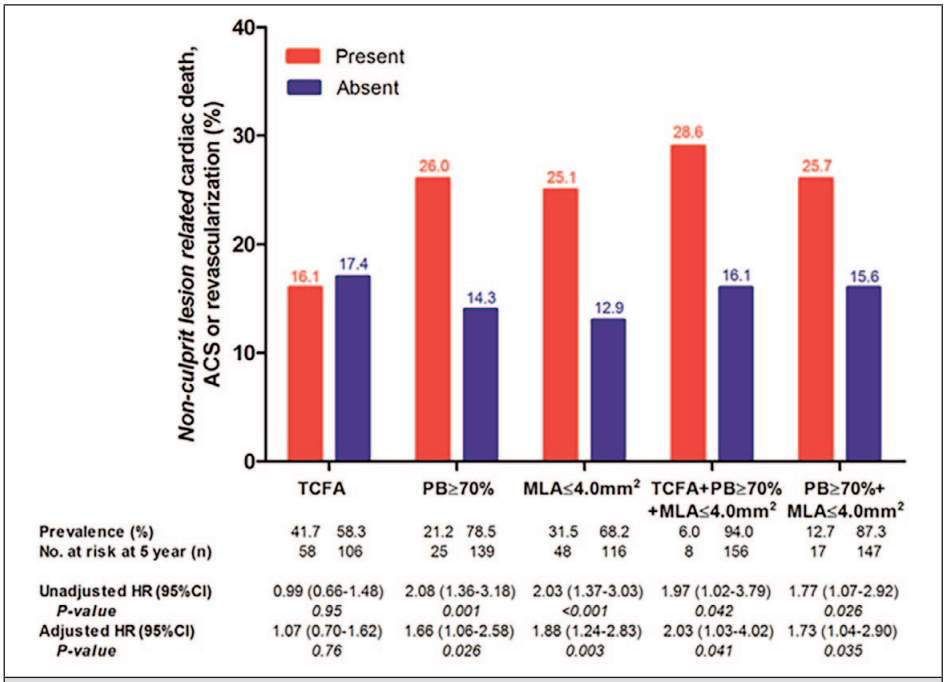

Figure 2. Association between (RF-)IVUS derived lesion characteristics and the composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization, after exclusion of culprit lesion-related events. Percentages are cumulative events rates estimated by the Kaplan-Meier method. Prevalence (%) in the footer refers to the prevalence of the specific (RF-)IVUS characteristic. P-values are obtained by the Log-rank test. Hazard ratios are estimated by univariate Cox regression analyses.

ACS, acute coronary syndrome; MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma

for the presence of a lesion with a plaque burden ≥70%, or its combination with a minimal luminal area ≤4.0 mm^2, as well as for segmental plaque burden with each 10 units increase in segmental plaque burden resulting in a 26% increase in risk for occurrence of the composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization after exclusion of culprit lesion-related events (adjusted HR: 1.26 per 10 units increase, 95% CI: 1.03-1.52, p=0.022) (Figure 2 and Table 3).

DISCUSSION

This 4.7-year follow-up of the ATHEROREMO-IVUS study demonstrated that a small luminal area and a large plaque burden, but not RF-IVUS-derived compositional plaque features on their own, are independent determinants of (non-culprit lesion-related) adverse cardiac events in patients with CAD. The increased risk associated with a minimal luminal area ≤4.0 mm^2 was not observed at 1-year follow-up,(15) whereas the prognostic value of plaque burden ≥70% was confirmed although statistical significance was not consistently present for all different composite endpoints. In contrast, the independent association between a TCFA lesion as an isolated characteristic and adverse outcome at 1-year did not persist during long-term follow-up. Still, patients with a TCFA lesion with a large plaque burden and/or a small luminal area were at increased risk.

In line with the PROSPECT study, we found that a lesion with a large plaque burden, small luminal area, or their combination with a TCFA lesion, predicted adverse cardiovascular events in patients with CAD during long-term follow-up. In contrast to the PROSPECT and Virtual histology Intravascular ultrasound in Vulnerable Atherosclerosis (VIVA) study, we did not find such an independent association for a TCFA lesion on its own.(13,14) However, the results of our study and the PROSPECT study cannot be directly compared since different definitions of study endpoints were used. In addition, PROSPECT only included patients admitted with ACS and the study endpoint was primarily driven by rehospitalizations.(13) Furthermore, in the VIVA study only univariable regression analysis was performed due to the small number of endpoints.(14) Importantly, in both the PROSPECT and VIVA study, (RF-)IVUS was applied in all three major coronary arteries, whereas in our study only one single non-stenotic non-culprit coronary artery segment was investigated.(13,14)

We consider several possible explanations for the inconsistent association between the presence of a TCFA lesion as an isolated characteristic and the risk of adverse cardiac events during short-term versus long-term follow-up. First, controversy exists about the ability of RF-IVUS to correctly discern and identify the thin-cap and necrotic core as individual components of a TCFA lesion, due to the limitations with respect to spatial resolution.(4,19) Second, the dynamic nature of TCFA lesions over time should be

appreciated, since it has been described that particularly (proximal) TCFA lesions with a large plaque burden heal less often and might have a greater tendency to rupture.(20) This may explain our finding that the presence of a TCFA lesion with a large plaque burden was associated with an increased risk for adverse cardiac events over 4.7-years of follow-up, whereas a TCFA lesion in itself was not. Third, previous studies have demonstrated that a lesion with a large plaque burden is a consistent and prevalent predictor for adverse cardiac outcome. However, whereas the atherosclerotic disease burden has been shown as a consistent and strong predictor of adverse cardiovascular events, no study has yet demonstrated that a TCFA lesion by itself independently predicts adverse cardiovascular outcome after adjustment for plaque burden and other potential confounders.(13,14,21,22)

Our current study suggests that a RF-IVUS-derived TFCA lesion only has long-term prognostic value if accompanied with other high-risk plaque features. Therefore, this study further adds to the discussion as to whether RF-IVUS offers incremental prognostic value to greyscale IVUS in terms of identification of high-risk coronary plaque phenotypes based on compositional features. In addition, our current study demonstrates for the first time that (RF-)IVUS plaque characteristics, as assessed in one non-stenotic segment of a non-culprit coronary artery, predicts adverse cardiovascular events in patients with CAD during long-term follow-up. A post-hoc analysis did not show heterogeneity in the HR estimates in patients with ACS versus SAP. Moreover, the large number of endpoints allowed for a separate analysis with exclusion of culprit-lesion related endpoint events, with results that remained essentially unchanged. This indicates that (RF-)IVUS-derived plaque characteristics, as identified in one non-culprit coronary artery segment, may reflect atherosclerotic vulnerability of the entire coronary tree.

Recently, we have demonstrated that the lipid core burden index, as assessed by NIRS in a single non-culprit coronary artery segment, predicts adverse cardiovascular outcome, independent of clinical characteristics and IVUS-derived segmental plaque burden, over 4 years in CAD patients referred for CAG.(23) In this context, a combined NIRS-IVUS catheter may improve the (long-term) prognostic value of intravascular imaging in patients with CAD.(24)

Limitations

Several study limitations warrant consideration. First, the number of TCFA lesions might be overestimated by RF-IVUS because of the limited spatial resolution with respect to the identification of the thin-cap of a TCFA lesion. Second, IVUS imaging was not repeated during follow-up. Therefore, we could not account for the potential dynamic nature of coronary lesions. It should also be noted that this study does not provide insight in how the individual lesion correlates to the adverse event. Third, the follow-up questionnaire was completed by 86% of the patients. Although for the majority of the remaining

patients follow-up information was retrieved from our local hospital records, we cannot fully exclude the possibility that loss to follow-up was in part selective. However, our study reflects daily clinical practice since patients admitted with both ACS and SAP were included. Besides, the current study represents a long-term study investigating the association between (RF-)IVUS-derived plaque characteristics and adverse cardiovascular outcome during 4.7-years of follow-up in patients with ACS or SAP, which represents the longest follow-up reported so far.

Conclusions

This study demonstrates that a small luminal area and a large plaque burden, and not RF-IVUS-derived compositional plaque features on their own, as assessed by (RF-)IVUS in *one* single non-stenotic segment of a non-culprit coronary artery, predict (non-culprit lesion-related) adverse cardiovascular outcome during long-term follow-up over 4.7-years in patients with CAD. In contrast, this study did not show a single isolated imaging parameter as derived by RF-IVUS to be of long-term independent prognostic value.

PERSPECTIVES

Core Clinical Competencies

Competency in Medical Knowledge

Whereas coronary angiography only yields a two-dimensional silhouette of the lumen, greyscale intravascular ultrasound (IVUS) and radiofrequency (RF-)IVUS have been shown to be able to identify high-risk coronary plaque characteristics within the coronary artery wall. Therefore, (RF-)IVUS may be useful to identify patients at increased risk of future adverse cardiovascular events. Identification of high-risk coronary plaque characteristics by invasive imaging may therefore improve risk stratification and management of patients with coronary artery disease.

Translational Outlook

Future studies should investigate whether a combined NIRS-IVUS catheter as an invasive imaging tool may improve risk prediction, as well as prevention and treatment of patients at increased risk of adverse cardiovascular outcome.

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Supplemental table 1. Associations of (RF-)IVUS characteristics and risk of MACE at 4.7-years of follow-up using two different models for multivariable adjustment.

	1-year follow-up data – adjusted model with 6 covariates HR (95% CI)	P-value	5-year follow-up data – adjusted model with 6 covariates HR (95% CI) †	P-value	5-year follow-up data – adjusted model with 11 covariates HR (95% CI) ‡	P-value
MACE						
TCFA	1.98 (1.09-3.60)	0.026	1.25 (0.90-1.73)	0.18	1.27 (0.91-1.77)	0.16
PB≥70%	2.90 (1.60-5.25)	<0.001	1.42 (0.99-2.05)	0.059	1.33 (0.92-1.93)	0.13
MLA≤4.0mm^2	1.23 (0.67-2.26)	0.50	1.51 (1.09-2.09)	0.014	1.49 (1.07-2.08)	0.020
TCFA+PB≥70%	–	–	1.80 (1.18-2.75)	0.007	1.73 (1.12-2.66)	0.013
TCFA+ MLA≤4.0mm^2	–	–	1.61 (1.01-2.57)	0.046	1.50 (0.93-2.44)	0.10
TCFA+PB≥70%+ MLA≤4.0mm^2	–	–	1.82 (1.02-3.26)	0.043	1.74 (0.97-3.13)	0.066
Segmental plaque burden (per 10 units increase)	–	–	1.17 (1.01-1.36)	0.040	1.15 (0.98-1.34)	0.079
Segmental plaque volume (per 100 units increase)	–	–	1.03 (0.92-1.16)	0.63	1.02 (0.90-1.14)	0.79

CI, confidence interval; HR, hazard ratio; IVUS, intravascular ultrasound; MACE, major adverse cardiovascular events; MLA, minimal luminal area; PB, plaque burden; RF, radiofrequency; TCFA, thin-cap fibroatheroma.

† Variables entered in the 6-covariate model were age, gender, diabetes mellitus, hypertension, history of percutaneous coronary intervention and indication for coronary angiography.

‡ Variables entered in the 11-covariate model were age, gender, diabetes mellitus, hypertension, history of percutaneous coronary intervention, indication for coronary angiography, dyslipidemia, history of myocardial infarction, history of coronary artery bypass grafting, history of peripheral artery disease and percutaneous coronary intervention performed at index.

CORONARY VULNERABILITY

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SYNTAX SCORE II PREDICTS LONG-TERM MORTALITY IN PATIENTS WITH ONE- OR TWO-VESSEL DISEASE

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ABSTRACT

Objective: SYNTAX score II (SSII) is a long-term mortality prediction model to guide the decision making of the heart-team between coronary artery bypass grafting or percutaneous coronary intervention (PCI) in patients with left main or three-vessel coronary artery disease. This study aims to investigate the long-term predictive value of SSII for all-cause mortality in patients with one- or two-vessel disease undergoing PCI.

Methods: A total of 628 patients (76% men, mean age: 61±10 years) undergoing PCI due to stable angina pectoris (43%) or acute coronary syndrome (57%), included between January 2008 and June 2013, were eligible for the current study. SSII was calculated using the original SYNTAX score website (www.syntaxscore.com). Cox regression analysis was used to assess the association between continuous SSII and long-term all-cause mortality. The area under the receiver-operating characteristic curve was used to assess the performance of SSII.

Results: SSII ranged from 6.6 to 58.2 (median: 20.4, interquartile range: 16.1-26.8). In multivariable analysis, SSII proved to be an independent significant predictor for 4.5-year mortality (hazard ratio per point increase: 1.10; 95% confidence interval: 1.07-1.13; $p<0.001$). In terms of discrimination, SSII had a concordance index of 0.77.

Conclusion: In addition to its established value in patients with left main and three-vessel disease, SSII may also predict long-term mortality in PCI-treated patients with one- or two-vessel disease.

INTRODUCTION

The SYNTAX score II (SSII) has been established as a long-term mortality prediction model to guide the decision making of the heart-team between coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) in patients with complex coronary artery disease (CAD).[1] It combines the original anatomical-based SYNTAX score, which grades the complexity of CAD in all coronary arteries, with the clinical baseline variables that have shown to be important predictors of 4-year all-cause mortality in the SYNTAX trial.[1]

SSII has been validated in large patient cohorts with left main or three-vessel disease. [1-3] However, the predictive performance of SSII on long-term mortality in patients with less complex CAD is currently unknown.

This study aims to investigate the long-term predictive value of SSII for all-cause mortality in patients with one- or two-vessel disease undergoing PCI.

METHODS

Study design and population

This study combines the populations of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis-intravascular ultrasound (ATHEROREMO-IVUS) study and the Integrated Biomarker and Imaging Study-3 (IBIS-3). [4, 5] Study designs and methods of ATHEROREMO-IVUS and IBIS-3 have been described in detail elsewhere.[4, 5] Baseline study procedures and inclusion criteria were similar and both studies were conducted at the Erasmus Medical Center, Rotterdam, The Netherlands. In brief, patients undergoing diagnostic coronary angiography (CAG) or PCI for an acute coronary syndrome (ACS) or stable angina pectoris (SAP) were included. During CAG, invasive imaging was performed in one non-culprit coronary artery segment. Subsequently, patients were followed-up on adverse cardiovascular events. Patient care was left at the discretion of the physician. During the first year after the index procedure, as per protocol, patients included in IBIS-3 received high dose rosuvastatin.

The medical ethics committee of the Erasmus MC approved both the ATHEROREMO-IVUS and IBIS-3 study. Both studies were performed in accordance with the declaration of Helsinki. All patients provided written informed consent for their participation in these studies, and compliance with the study protocols, including long-term follow-up. The ATHEROREMO-IVUS study is registered in ClinicalTrials.gov, number NCT01789411, and the IBIS-3 study is registered in The Netherlands trial register, number NTR2872.

SYNTAX score II

The anatomical-based SYNTAX score was determined (pre-PCI) for every coronary angiogram taken at study entry, by a trained analyst blinded for patient characteristics and outcome using the SYNTAX Score Calculator (www.syntaxscore.com). The calculation of the anatomical-based SYNTAX score has been described in detail previously.[6] In brief, the complete coronary tree is divided in 16 segments and assessed for lesions producing 50% or more luminal obstruction. Every segment has a pre-specified corresponding weighing factor which, in case of a significant lesion, is added to the SYNTAX score by the SYNTAX Score Calculator. Moreover, other variables (i.e. calcification or lesion length) that reflect the complexity of a patient's CAD and, thus, the complexity of treatment are assessed and taken into account in the SYNTAX score. Eventually, the SYNTAX score is composed of these total points summed, and reflects the complexity of a patient's CAD. As previously applied in other all-comers and ST-segment elevation myocardial infarction (STEMI) populations, lesions caused by in-stent restenosis were treated as de novo lesions.[7-9] Total occlusions were scored as occlusions of unknown duration, as the analyst was blinded for all patient information.[10]

Subsequently, data on the baseline variables age, gender, creatinine clearance (CRCL), left ventricular ejection fraction (LVEF), peripheral vascular disease and chronic obstructive pulmonary disease was collected for the calculation of SSII. We used the original SYNTAX Score II Calculator (www.syntaxscore.com) to obtain all SSII values. The algorithm of the SSII calculation has been described in detail elsewhere.[1]

Study endpoint

The primary endpoint was all-cause mortality. Vital status of the patients was obtained from municipal civil registries.

Statistical analysis

The distribution of continuous variables was examined for normality with the Kolmogorov-Smirnov test. ANOVA or Kruskal-Wallis test were used for multiple group comparison of continuous variables. Categorical variables were compared using the Pearson Chi-square test.

Data for most of the variables used for the calculation of SSII were complete. However, creatinine, required for the calculation of CRCL, was available in 92.8% of the patients. LVEF was available in 72.0% of the patients and categorized as good (LVEF≥50%), moderate (LVEF 40-49%) and poor (LVEF<40%).[11] Because LVEF was reported qualitatively, a value of 50% was used for category good, 44.5% for category moderate and 35% for category poor for the calculation of SSII. Multiple imputation technique was used to impute the missing data of creatinine and LVEF. Ten imputed data sets were generated.

Analyses were conducted for both the complete dataset as the imputed datasets, which showed similar results.

Long-term cumulative incidences of all-cause mortality, categorized by SSII in tertiles, were compared with the log-rank test. Cox regression analysis was used to assess the association between continuous SSII and long-term all-cause mortality. Patients that were lost to follow-up were censored at the date of last contact. Based on existing literature, variables known to be associated with mortality and not part of the SSII (diabetes mellitus, hypertension, smoking, previous PCI and indication for coronary angiography) were entered in a multivariable Cox model. Since our study population also includes STEMI-patients and the use of SSII has been validated in stable patients, a subgroup analysis was performed in patients with SAP only, to compare the results of the total study population with the results found in patients with SAP only. In terms of discrimination, the area under the receiver-operating characteristic (ROC) curve was assessed. All statistical test were two-sided with a type I error level of 0.05. Analyses were performed with IBM SPSS Statistics version 21.0.

RESULTS

A total of 628 patients (76% men, mean age: 61 ±10 years) undergoing PCI due to SAP (43%) or ACS (57%), included between January 2008 and June 2013, were eligible for the current study (Table 1). SSII ranged from 6.6 to 58.2 (median: 20.4, IQR: 16.1-26.8). All-cause mortality occurred in 44 patients (7.0%) during a median follow-up of 4.5 (IQR: 3.4-4.9) years. Patients with a high SSII were older, had a higher prevalence of diabetes mellitus, hypertension, hypercholesterolemia and COPD, and more frequently had a history of renal insufficiency or heart failure than patients with a mid or low SSII.

Cumulative incidence of all-cause mortality categorized by SSII in tertiles is shown in Figure 1. The long-term cumulative incidence of all-cause mortality of patients with a high SSII showed to be significantly higher than for patients with a mid or low SSII. No statistically significant difference was found between the cumulative incidence of all-cause mortality of patients with a mid versus low SSII value.

In the multivariable Cox model (Table 2), SSII proved to be an independent significant predictor for 4.5-year mortality (hazard ratio [HR] per point increase: 1.10; 95% confidence interval [CI]: 1.07-1.13). For SAP patients only, results were similar to the total study population (HR: 1.06; 95%CI: 1.07-1.11). In terms of discrimination, SSII had a concordance index (c-index) of 0.77 (95%CI: 0.69-0.84) (Figure 2).

Table 1. Baseline characteristics

	SSII ≤17	17< SSII ≤24	SSII >24	
	(n = 209)	(n = 210)	(n = 209)	p-value
Clinical characteristics				
Age - yrs, ± sd	52.9 ± 7.8	61.5 ± 8.1	69.0 ± 9.2	<0.001
Men, n(%)	204 (97.6)	163 (77.6)	109 (52.2)	<0.001
Diabetes mellitus, n(%)	28 (13.4)	40 (19.0)	49 (23.4)	0.051
Hypertension, n(%)	91 (34.5)	112 (53.3)	134 (64.1)	<0.001
Hypercholesterolemia, n(%)	96 (45.9)	120 (57.1)	127 (60.8)	0.025
Current smoking, n(%)	88 (42.3)	60 (28.6)	47 (22.5)	<0.001
Previous MI, n(%)	55 (26.3)	57 (27.1)	64 (30.6)	0.48
Previous PCI, n(%)	58 (27.8)	65 (31.0)	60 (28.7)	0.74
Previous CVA, n(%)	10 (4.8)	10 (4.8)	18 (8.6)	0.16
History of PAD, n(%)	0 (0.0)	0 (0.0)	46 (22.0)	<0.001
History of renal insufficiency, n(%)	6 (2.9)	4 (1.9)	20 (9.6)	<0.001
History of heart failure, n(%)	1 (0.5)	2 (1.0)	10 (4.8)	0.003
COPD, n(%)	1 (0.5)	9 (4.3)	23 (11.0)	<0.001
Serum creatinine - µmol/L, ± sd	77.3 ± 13.2	74.8 ± 17.3	84.6 ± 28.3	<0.001
Creatinine clearance - ml/min, ± sd	127.8 ± 31.8	111.9 ± 34.3	80.1 ± 27.8	<0.001
LVEF, n (%)				<0.001
Good LVEF ≥50%	189 (90.4)	156 (74.2)	136 (65.1)	
Moderate LVEF 40-49%	20 (9.6)	54 (25.8)	65 (31.1)	
Poor LVEF <40%	0 (0.0)	0 (0.0)	8 (3.8)	
Angiographic characteristics				
Indication for angiography, n(%)				0.16
Acute MI	77 (36.8)	64 (30.6)	51 (24.4)	
Unstable angina	58 (27.8)	57 (27.3)	61 (29.2)	
Stable angina	74 (35.4)	99 (47.4)	97 (46.4)	
Coronary artery disease, n(%)				0.007
1-vessel disease	146 (69.9)	118 (56.5)	115 (55.0)	
2-vessel disease	63 (30.1)	92 (43.5)	94 (45.0)	
Median SS [IQR]	5.0 [3.0-9.0]	9.0 [5.0-13.5]	9.0 [5.0-15.0]	<0.001

CI: confidence interval; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; IQR: inter quartile range; LVEF: left ventricular ejection fraction; MI: Myocardial infarction; PAD: Peripheral artery disease; PCI: Percutaneous coronary intervention; sd: standard deviation; SS: SYNTAX score; SSII: SYNTAX score II; yrs: years

DISCUSSION

In this study, we validated for the first time the use of SSII for prediction of long-term mortality in a large PCI-treated patient population with one- or two-vessel disease.

Table 2. Prediction of long-term mortality

Total population (n=628)			*SAP patients only (n=270)*		
	Unadjusted HR (95%CI)	p value		Unadjusted HR (95%CI)	p value
SSII	1.09 (1.07-1.12)	<0.001	SSII	1.05 (1.00-1.10)	0.050
	Adjusted HR (95%CI)			Adjusted HR (95%CI)	
SSII	1.10 (1.07-1.13)	<0.001	SSII	1.06 (1.07-1.11)	0.037
Smoking	1.01 (0.52-1.98)	0.97	Smoking	1.52 (0.48-4.82)	0.48
Diabetes mellitus	1.60 (0.79-3.24)	0.19	Diabetes mellitus	1.56 (0.52-4.62)	0.43
Hypertension	0.88 (0.46-1.68)	0.70	Hypertension	0.59 (0.21-1.71)	0.33
Previous PCI	1.09 (0.54-2.18)	0.82	Previous PCI	0.59 (0.20-1.75)	0.35
Indication for CAG, SAP	0.59 (0.30-1.15)	0.12			

SSII incorporates the anatomical Syntax score, age, gender, creatinine clearance, left ventricular ejection fraction, peripheral vascular disease and chronic obstructive pulmonary disease.
CAG: coronary angiography; CI: confidence interval; HR: hazard ratio; PCI: percutaneous coronary intervention; SAP: stable angina pectoris; SSII: Syntax score II

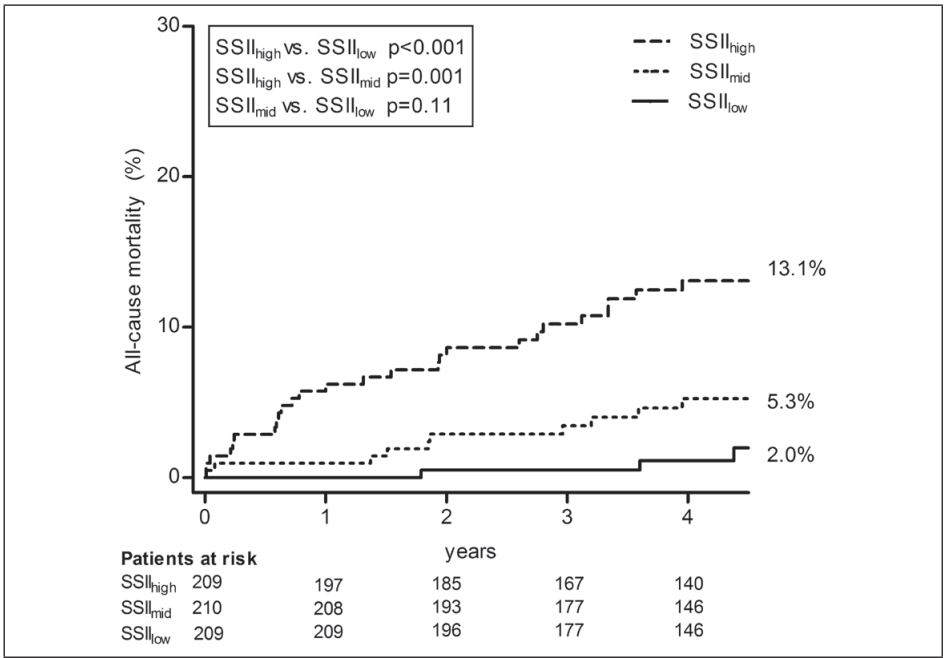

Figure 1. Cumulative incidence of all-cause mortality at 4.5 years
SSII is divided in tertiles with cut-off points 17 and 24 to compare the cumulative all-cause mortality proportions between patients with a low, mid or high SSII value.
SSII: Syntax score II

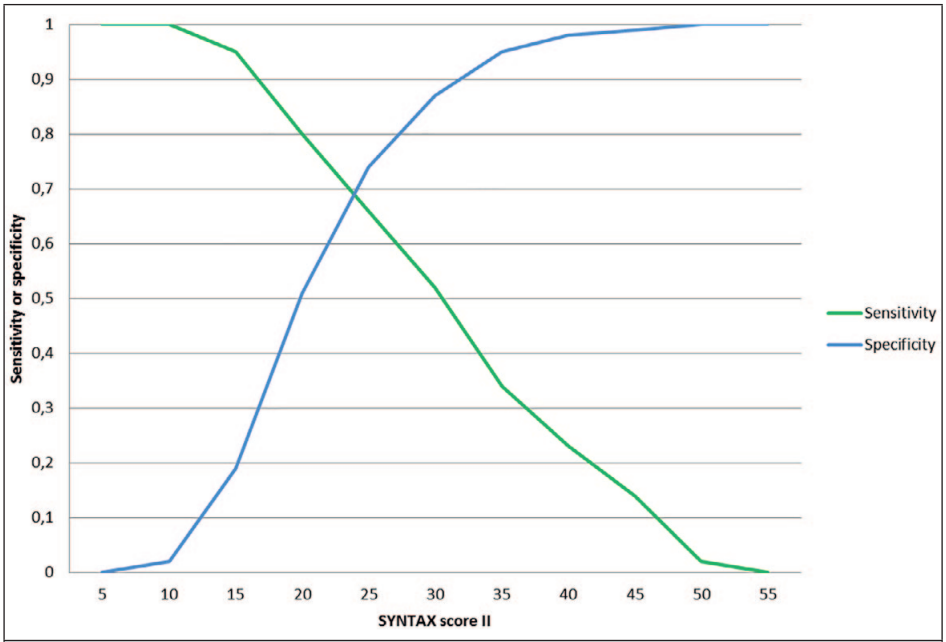

Figure 2. Sensitivity and specificity of SSII for the long-term prediction of all-cause mortality

SSII demonstrated to be an independent predictor for 4.5-year all-cause mortality in multivariable analysis. Moreover, in terms of discrimination, SSII had a c-index of 0.77 which is in line with the internally and externally validated c-indices of 0.73 and 0.72, respectively, of SSII in the SYNTAX trial for CABG or PCI-treated patients with left main or three-vessel disease.[1] It is also in line with the c-index (0.75) of SSII found in a study of patients with left main or three-vessel disease treated with only PCI.[3]

Although other known predictors of all-cause mortality in patients with CAD which are not part of SSII, such as diabetes mellitus, hypertension and prior PCI, were entered in the multivariable Cox model, SSII demonstrated to be the only significant predictor for 4.5-year all-cause mortality. Diabetes mellitus is a well-known predictor for adverse outcome in patients treated with PCI.[12] However, our findings imply that SSII incorporates enough relevant clinical prognostic variables to predict long-term all-cause mortality in patients with one- or two-vessel disease. Recently, the performance of SSII has been compared in diabetic patients versus non-diabetic patients with multi-vessel or left main disease undergoing PCI.[13] The SSII showed to have a good discriminative ability in both patient groups, independent of diabetic status. It may be hypothesized that other clinical variables incorporated in the SSII, such as CRCL, sufficiently reflect the influence of diabetes mellitus. In this respect, a previous study has demonstrated that kidney disease is of greater importance than diabetes mellitus for risk prediction of adverse outcome in patients with CAD.[14]

SSII has been developed for individual risk assessment using a continuous scale to overcome the limitations of categorized risk scores. Our study validates the use of SSII in patients with one- or two-vessel disease, demonstrating a similar discrimination as previously reported in left main or three-vessel disease.

Limitations

In our study, the calculation of anatomical-based SYNTAX score for SSII included small vessels of at least 1.5mm and intermediate stenosis causing luminal obstruction of <70%, as instructed by the SYNTAX trial.[6] However, as recently observed in prospective registries, intermediate stenosis and small vessels <2.0 mm may not have additive predictive value for the prognosis of late mortality.[15-17] Hence, SSII calculated when only including severe stenosis of >70% in vessels of at least 2 mm, may even more accurately predict late mortality than currently observed in our study.

Further, the modest reproducibility of the anatomical-based SYNTAX score has to be acknowledged.[18] However, since our study population with one- or two-vessel disease had a relatively low angiographic burden, we expected a fair reproducibility of the anatomical-based SYNTAX score. To assess the reproducibility, a second experienced analyst repeated the anatomical-based SYNTAX score analysis in a representative random sample, blinded for patient information and previously scored SXscores. Cohen's kappa was 0.91, which indicated a good interobserver agreement. Furthermore, since SSII is used in a continuous manner and incorporates both anatomical as well as clinical variables, SSII offers higher accuracy than the original anatomical-based SYNTAX score.[3]

In addition, our single-center study needs external validation. As expected, the median SSII score in our population was lower than in the original SSII report and further research is required to investigate the relation between the actual SSII and corresponding event rate in one- or two-vessel disease.

Conclusion

This study validates the predictive performance of SSII in patients with one- or two-vessel disease indicating that, in addition to its known value in patients with left main or three-vessel disease, SSII may also offer accurate risk prediction in patients with less complex CAD.

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CORONARY VULNERABILITY

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RELATION OF C-REACTIVE PROTEIN TO CORONARY PLAQUE CHARACTERISTICS ON GRAYSCALE, RADIOFREQUENCY INTRAVASCULAR ULTRASOUND, AND CARDIOVASCULAR OUTCOME

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ABSTRACT

The relation between C-reactive protein (CRP) and coronary atherosclerosis is not fully understood. This study aims to investigate the associations between high-sensitivity CRP, coronary plaque burden and presence of high-risk coronary lesions as measured by intravascular ultrasound (IVUS), as well as 1-year cardiovascular outcome. Between 2008 and 2011, grayscale and virtual histology IVUS imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for acute coronary syndrome (ACS) or stable angina pectoris. Primary endpoint consisted of 1-year major adverse cardiac events (MACE), defined as all-cause mortality, ACS or unplanned coronary revascularization. After adjustment for established cardiac risk factors, baseline CRP levels were independently associated with higher coronary plaque burden (p=0.002) and plaque volume (p=0.002) in the imaged coronary segment. CRP was also independently associated with presence of large lesions (plaque burden ≥70%; p=0.030), but not with presence of stenotic lesions (minimal luminal area ≤4.0mm^2; p=0.62) or IVUS virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesions (p=0.36). Cumulative incidence of 1-year MACE was 9.7%. CRP levels >3mg/L were independently associated with a higher incidence of MACE (HR2.17, 95%CI 1.01-4.67, p=0.046) and of all-cause mortality and ACS only (HR3.58, 95%CI 1.04-13.0, p=0.043), when compared to CRP levels <1mg/L. In conclusion, in patients undergoing coronary angiography, high-sensitivity CRP is a marker of coronary plaque burden, but is not related to the presence of VH-TCFA lesions and stenotic lesions. CRP levels of >3 mg/L are predictive for adverse cardiovascular outcome at 1 year.

INTRODUCTION

C-reactive protein (CRP) is a prognostic marker of cardiovascular outcome in patients with stable coronary artery disease and patients with acute coronary syndrome (ACS).[1-3] Although CRP has also been postulated to reflect the extent of coronary atherosclerosis as well as plaque vulnerability, these relations are not yet fully understood.[4] Previous studies have only shown weak associations between CRP and the extent of coronary artery disease on angiography and the degree of coronary calcification on computed tomography.[3,5,6] Furthermore, the associations between CRP and the presence of high-risk vulnerable plaque morphology has not been investigated yet.[7] Grayscale intravascular ultrasound (IVUS) imaging of the coronary arteries allows for accurate measurement of coronary plaque burden and plaque volume, as well as identification of large or stenotic lesions.[8-10] Additionally, IVUS virtual histology (IVUS-VH) (i.e. analysis of IVUS radiofrequency backscatter), allows tissue characterization and for identification of virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesions.[8-13] This study aims to investigate the associations between high sensitivity CRP, coronary plaque burden and presence of high-risk coronary lesions (i.e. VH-TCFA lesions, lesions with large plaque burden, and stenotic lesions) as measured by grayscale and radiofrequency IVUS, as well as 1-year cardiovascular outcome.

METHODS

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described in detail elsewhere.[9,14] 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. 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 in ClinicalTrials.gov, number NCT01789411.

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples stored at temperature of –80°C within 2 hours after blood collection. CRP was measured in the stored serum samples (n=576) using a immunoturbidimetric high sensitivity assay (Roche Diagnostics Ltd., Rotkreuz, Switzerland) on the Cobas 8000 modular analyzer platform (Roche Diagnostics Ltd., Rotkreuz, Switzerland). The diagnostic range of this assay is 0.3-350 mg/L with a coefficient of variation of 1.3%

at a mean value of 2.63 mg/L. In 5 patients, serum samples were not available for CRP measurement.

Following the standard coronary angiography procedure, IVUS imaging of the most proximal part 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 grayscale 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 (median interslice distance, 0.40 mm). 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 (Figure 1). A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Using IVUS-VH, the composition of the atherosclerotic lesions was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.[12] Three types of high-risk lesions were identified: 1. VH-TCFA lesion, defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen in at least 3 consecutive frames; 2. lesion with large plaque burden, defined as a lesion with a plaque burden of ≥70% in at least 3 consecutive frames; 3. stenotic lesion, defined as a lesion with a minimal luminal area of ≤4.0 mm^2 in at least 3 consecutive frames (Figure 1).[8-11,13]

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 (i.e. discharge letters and coronary angiogram) whenever necessary. 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) were classified into both categories. When information was not sufficient to classify an event as either culprit lesion related or non-culprit lesion related,

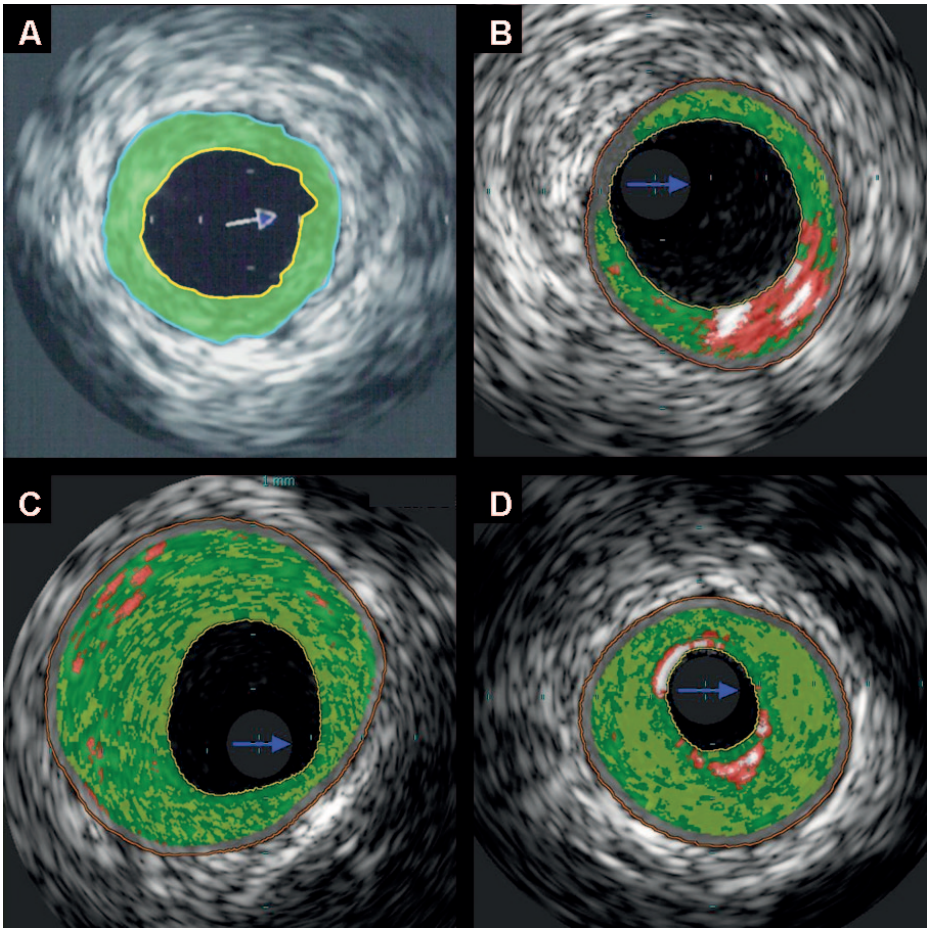

Figure 1. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound

A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma lesion, defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. C: Lesion with plaque burden of ≥70%. D: Lesion with a minimal luminal area of ≤4.0 mm^2.

the event was classified as indeterminate. The endpoints were adjudicated by a clinical event committee that had no knowledge of the CRP and IVUS data.

The primary clinical endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. 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.[15] Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). The secondary end-

point was defined as the composite of all-cause mortality or ACS. Additional analyses were performed on non-culprit lesion-related and indeterminate events only (definite culprit lesion-related events were excluded in these analyses).

The distributions of the continuous variables, including CRP levels and the IVUS parameters, were tested for normality by visual examination of the histogram. CRP was not normally distributed and was therefore ln-transformed when analyzed as continuous variable. CRP levels were also categorized as low (<1 mg/L), average (1-3 mg/L) or high (>3 mg/L) according to the recommendations from the Centers for Disease Control and Prevention and the American Heart Association.[16] Categorical variables are presented as numbers and percentages. We examined associations of CRP concentration with plaque burden, plaque volume and presence of high-risk coronary lesions. Plaque volume was normalized for the imaged segment length (normalized plaque volume = plaque volume / imaged segment length * median segment length of study population). To test for trends, we used linear regression and logistic regression analyses with continuous ln-transformed CRP concentration as independent variable. Z-test for heterogeneity was performed to test for differences in effect estimates between patients admitted with and without ACS. In multivariable analyses, the variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, peripheral artery disease, history of PCI, statin use at time of hospital admission and indication for coronary angiography were considered as potential confounders (specifically: the variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia and smoking represent the traditional cardiac risk factors; the variables peripheral artery disease and history of PCI represent the presence of clinically manifest atherosclerosis; statin use may modulate baseline CRP levels; and the different indications for coronary angiography represent different patient risk classes) and were therefore entered into the each multivariate model.

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. Cox proportional hazards regression analyses were performed to evaluate the associations between CRP and study endpoints. The final results are presented as crude and adjusted hazard ratios (HR) with 95% confidence interval (95% CI). 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

Mean age of the patients was 61.5 ± 11.3 years, 76% were men, and 55% had ACS (Table 1). Mean plaque burden in the imaged coronary segment was 38.2% ± 11.5% and mean

Table 1. Baseline characteristics

Variable	Total (n=576)	C-reactive protein (mg/L) <1 (n=172)	1-3 (n=185)	>3 (n=219)	P
Age (years)	61.5 ± 11.3	61.0 ± 10.2	61.3 ± 12.0	62.1 ± 11.6	0.59
Men	435 (75.5%)	140 (81.4%)	143 (77.3%)	152 (69.4%)	0.019
Diabetes mellitus	99 (17.2%)	28 (16.3%)	30 (16.2%)	41 (18.7%)	0.75
Hypertension[a]	300 (52.1%)	93 (54.1%)	95 (51.4%)	112 (51.1%)	0.84
Hypercholesterolemia[a]	320 (55.6%)	107 (62.2%)	102 (55.1%)	111 (50.7%)	0.082
Smoker	167 (29.0%)	34 (19.8%)	56 (30.3%)	77 (35.2%)	0.003
Positive family history[b]	300 (52.1%)	99 (57.6%)	91 (49.2%)	110 (50.2%)	0.20
Previous MI	183 (31.8%)	52 (30.2%)	61 (33.0%)	70 (32.0%)	0.85
Previous coronary intervention	186 (32.3%)	62 (36.0%)	66 (35.7%)	58 (26.5%)	0.065
Previous coronary bypass	18 (3.1%)	5 (2.9%)	8 (4.3%)	5 (2.3%)	0.49
Previous stroke	26 (4.5%)	8 (4.7%)	8 (4.3%)	10 (4.6%)	0.99
Peripheral artery disease	36 (6.2%)	5 (2.9%)	15 (8.1%)	16 (7.3%)	0.091
History of renal insufficiency	32 (5.6%)	12 (7.0%)	9 (4.9%)	11 (5.0%)	0.62
History of heart failure	19 (3.3%)	6 (3.5%)	7 (3.8%)	6 (2.7%)	0.83
High sensitivity CRP (mg/L)	2.1 [0.9-5.4]				
Indication for coronary angiography					<0.001
ACS	314 (54.5%)	71 (41.3%)	90 (48.6%)	153 (69.9%)	
ST-elevation MI	164 (28.5%)	45 (26.2%)	54 (29.2%)	65 (29.7%)	
Non-ST-elevation ACS	150 (26.0%)	26 (15.1%)	36 (19.5%)	88 (40.2%)	
Stable angina pectoris	262 (45.5%)	101 (58.7%)	95 (51.4%)	66 (30.1%)	
Number of narrowed coronary arteries					0.71
None	42 (7.3%)	14 (8.1%)	10 (5.4%)	18 (8.2%)	
1	306 (53.1%)	96 (55.8%)	93 (50.3%)	117 (53.4%)	
2	167 (29.0%)	46 (26.7%)	61 (33.0%)	60 (27.4%)	
3	61 (10.6%)	16 (9.3%)	21 (11.4%)	24 (11.0%)	
PCI performed	507 (88.0%)	153 (89.0%)	163 (88.1%)	191 (87.2%)	
Imaged coronary artery					0.14
Left anterior descending	207 (35.9%)	49 (28.5%)	74 (40.0%)	84 (38.4%)	
Left circumflex	193 (33.5%)	63 (36.6%)	55 (29.7%)	75 (34.2%)	
Right coronary artery	176 (30.6%)	60 (34.9%)	56 (30.3%)	60 (27.4%)	
Segment length (mm)	44.3 [33.8-55.4]	44.6 [36.4-54.3]	42.9 [32.5-54.6]	44.4 [32.0-56.3]	0.67

Data are presented as mean ± standard deviation or as median [interquartile range].

[a] Presence of hypertension and hypercholesterolemia were defined as a clinical diagnosis of these conditions as reported by the treating physician in the medical chart.

[b] Patient-reported positive family history of ischemic heart disease.

ACS, acute coronary syndrome; CRP, C-reactive protein; MI, myocardial infarction; PCI, percutaneous coronary intervention.

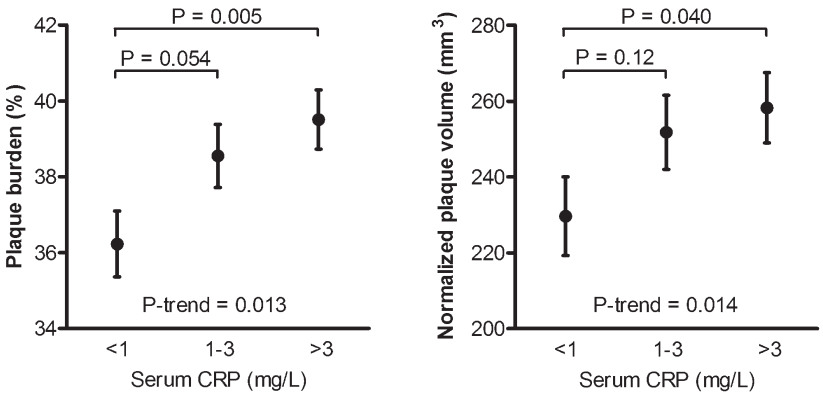

Figure 2. Association of CRP with coronary plaque burden and plaque volume of imaged coronary segment
CRP, C-reactive protein.

normalized plaque volume was 248 ± 136 mm^3. A total of 241 (42%) patients had ≥1 VH-TCFA lesion, 124 (22%) patients had ≥1 lesion with large plaque burden (plaque burden ≥70%) and 181 (31%) patients had ≥1 stenotic lesion (minimal luminal area ≤4.0 mm^2).

Higher CRP levels were associated with higher mean coronary plaque burden (p for trend = 0.013) and higher mean normalized plaque volume (p for trend = 0.015) in the imaged coronary segment (Figure 2). Higher CRP levels showed a tendency towards an association with the presence of lesions with large plaque burden (plaque burden ≥70%; p for trend = 0.093), while CRP was not associated with the presence of VH-TCFA lesions (p for trend = 0.36) or stenotic lesions (minimal luminal area ≤4.0 mm^2; p for trend = 0.62) on IVUS (Figure 3). There was no heterogeneity between ACS patients and patients with stable angina (heterogeneity on association with plaque burden p=0.45; plaque volume

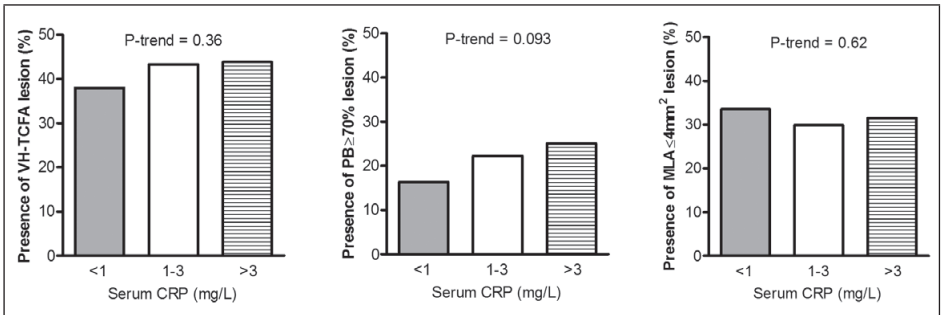

Figure 3. Association with presence of high risk coronary lesions
CRP, C-reactive protein; MLA, minimal luminal area; PB, plaque burden; VH-TCFA, virtual histology-derived thin-cap fibroatheroma.

p=0.71; lesions with large plaque burden p=0.21; VH-TCFA lesions p=0.70; stenotic lesions p=0.99) (Supplemental table 1). After adjustment for established cardiovascular risk factors, statin use, and the indication for coronary angiography, higher CRP levels remained significantly associated with higher plaque burden (per SD increase in ln-transformed CRP: β 1.49, 95% CI 0.55-2.43, p for trend = 0.002), plaque volume (per SD increase in ln-transformed CRP: β 0.080, 95% CI 0.030-0.131, p for trend = 0.002) and presence of lesions with large plaque burden (plaque burden ≥70%; OR per SD increase in CRP 1.27, 95% CI 1.02-1.58, p for trend = 0.030).

Vital status at 1-year follow-up could be acquired for 574 (99.7%) patients. Response rate of the questionnaires that were sent to all living patients was 92.4%. After 1 year of follow-up, 56 patients had experienced a MACE (Table 2). The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year MACE (primary endpoint) were 0.9%, 5.6%, and 9.7%, respectively. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year composite of death or ACS were 0.9%, 3.8%, and 5.6%, respectively.

In univariable analysis, higher CRP levels were associated with a higher incidence of MACE during follow-up (>3 vs. <1 mg/L: 11.9% vs. 5.8%, HR 2.11, 95%CI 1.02-4.38, p=0.044; 1-3 vs <1 mg/L: 10.8% vs. 5.8, HR 1.92, 95%CI 0.90-4.10, p=0.092) (Table 3, Figure 4). There was no heterogeneity in the hazard ratio estimate between ACS patients and patients with stable angina (Supplemental table 2). Higher CRP levels were also associated with the composite of death or ACS only (>3 vs <1 mg/L: 8.7% vs. 1.7%, HR 5.13, 95%CI 1.52-17.3, p=0.009; 1-3 vs <1 mg/L: 5.4% vs. 1.7%, HR 3.14, 95%CI 0.86-11.4, p=0.082). After adjustment for established cardiovascular risk factors, statin use and the indication for coronary angiography, CRP levels of >3 mg/L remained independently

Table 2. Incidence of major adverse cardiac events (n=56)

Variable	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 major adverse cardiac events	11	27	18	45[b]	56[a]
Death from any cause	1	1	16	17	18
Definite cardiac or unexplained death	1	1	6	7	8
Acute coronary syndrome	3	9	2	11	14
Myocardial infarction	2	3	2	5	7
Unplanned coronary revascularization	7	17	0	17	24
Composite of death or acute coronary syndrome	4	10	18	28[b]	32[b]

[a] Primary endpoint
[b] Secondary endpoint

Table 3. Prediction of cardiovascular outcome

Variable	Unadjusted HR (95%CI)	P	Adjusted[a] HR (95%CI)	P
MACE				
CRP 1-3 vs <1 mg/L	1.92 (0.90-4.10)	0.092	1.75 (0.80-3.81)	0.16
CRP >3 vs <1 mg/L	2.11 (1.02-4.38)	0.044	2.17 (1.01-4.67)	0.046
Composite of death or ACS				
CRP 1-3 vs <1 mg/L	3.14 (0.86-11.4)	0.082	2.23 (0.59-8.37)	0.24
CRP >3 vs <1 mg/L	5.13 (1.52-17.3)	0.009	3.68 (1.04-13.0)	0.043

[a] Adjusted for age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, peripheral artery disease, history of percutaneous coronary intervention, statin use and indication for coronary angiography.

ACS, acute coronary syndrome; CRP, C-reactive protein; MACE, major adverse cardiac event.

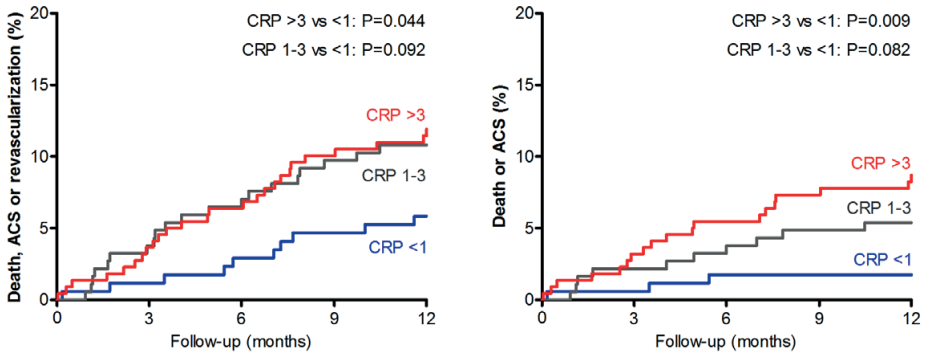

Figure 4. Prediction of cardiovascular outcome
ACS, acute coronary syndrome; CRP, C-reactive protein.

predictive for higher MACE rate (HR 2.17, 95%CI 1.01-4.67, p=0.046) and for the composite of death or ACS only (HR 3.58, 95%CI 1.04-13.0, p=0.043).

Additional analyses were performed on non-culprit lesion-related and indeterminate events only (definite culprit lesion-related events were excluded in these analyses). Although statistical significance disappeared for some associations because of the lower statistical power (less events), the estimates of the associations with non-culprit lesion-related and indeterminate events only were materially the same (Supplemental table 3).

DISCUSSION

This study investigated the associations of circulating CRP concentration with extent of coronary atherosclerosis, the presence of high-risk lesions, and the risk of adverse

cardiovascular outcome in patients who underwent coronary angiography for ACS or stable angina pectoris. The present study is the first large study that investigated the relation between high sensitivity CRP and coronary plaque characteristics using IVUS-VH. The main findings are that high CRP concentrations were associated with a higher coronary plaque burden and the presence of large lesions, but not with presence of VH-TCFA and stenotic lesions on grayscale IVUS and IVUS-VH.

Many epidemiologic studies have shown associations between elevated serum CRP concentrations and the risk of recurrent cardiovascular events among patients with established coronary artery disease, and the incidence of first cardiovascular events among individuals with cardiovascular risk factors.[16,17] In line with these findings, this study demonstrates that baseline CRP levels were predictive of a higher rate of cardiovascular events during the first year after coronary angiography in patients with known coronary artery disease.

CRP is hypothesized to reflect the extent of underlying atherosclerosis.[16,17] However, previous studies only found a weak (or even no) association with the extent of coronary artery disease on angiography and the degree of coronary artery calcification on computed tomography.[3,5,18] IVUS imaging may provide more accurate measures of the extent of coronary atherosclerosis. Our results support the hypothesis that serum CRP levels reflect the presence and extent of underlying atherosclerosis.

Other studies have suggested that CRP is a marker of plaque instability and plaque rupture.[19] This hypothesis was primarily based on the fact that CRP was found to be elevated in patients with ACS and that it displayed prognostic value for cardiovascular outcome. A population-based study also showed that high serum CRP levels were associated with the presence of mixed calcified arterial plaques on coronary computed tomography angiography, suggesting an association with plaque vulnerability.[20] In contrast, previous large studies showed conflicting results regarding to a direct pathogenic role of CRP in development of plaque vulnerability.[7,21] In the present study, we did not find an association between serum CRP and the presence of VH-TCFA lesions. A plausible explanation for our finding that CRP is still predictive for coronary events may be that CRP has a role in the evolution of stable coronary plaque to unstable plaque.[22] Furthermore, a substantial part of ACS cases are not attributable to plaque rupture, but to plaque erosion due to endothelial inflammation.[23] CRP may have a role in such endothelial inflammation as well.[24]

Patients with ACS had higher CRP levels than those with stable angina pectoris. Nevertheless, the distribution of CRP of both groups largely overlapped, so that the same standard cut-off values for CRP (<1, 1-3 and >3 mg/L) could be used for both groups. Although there was no heterogeneity on the associations with plaque characteristics and cardiovascular outcome between ACS patients and patients with stable angina pectoris, it should be acknowledged that heterogeneity is difficult to detect with the relatively small number endpoints in this study.

Some limitations of this study need to be acknowledged. Firstly, a single non-culprit coronary vessel was imaged in this study. High risk lesions (e.g. VH-TCFA lesions and stenotic lesions) elsewhere in the coronary tree could not be detected in our study. This may have lead to an underestimation of the association between CRP and the presence of high risk lesions in the coronary tree. Secondly, the spatial resolution of IVUS-VH (150μm) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65μm).[25] Therefore, IVUS-VH tends to over-estimate the number of thin-cap fibroatheroma lesions. Nevertheless, the presence of VH-TCFA lesions has been shown to have prognostic information.[8-10] Thirdly, repeat intracoronary imaging with IVUS was not performed. Therefore, the dynamic nature of coronary artery lesion morphology could not be investigated. Finally, the number of endpoints was relatively small. Consequently, we may have lacked statistical power to detect small effect sizes (e.g. in presence of VH-TCFA lesions). Furthermore, we were not able to evaluate whether adding CRP to a prognostic model with conventional risk factors would result in improved risk prediction and discrimination.

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SUPPLEMENTAL TABLES

Supplemental table 1. Associations between C-reactive protein and coronary plaque characteristics stratified by patients with acute coronary syndrome and patients with stable coronary artery disease

	Total study population	ACS patients	Stable CAD patients	P for heterogeneity
Plaque burden	β 0.80 (0.17 ; 1.44) p=0.013	β 1.31 (0.42 ; 2.20) p=0.004	β 0.81 (–0.13 ; 1.76) p=0.094	0.45
Normalized plaque volume (ln-transformed)	β 0.042 (0.008 ; 0.075) p=0.014	β 0.047 (0.001 ; 0.094) p=0.046	β 0.060 (0.009 ; 0.111) p=0.022	0.71
≥1 VH-TCFA lesion	OR 1.05 (0.94-1.18) p=0.36	OR 1.01 (0.87-1.18) p=0.91	OR 1.06 (0.88-1.26) p=0.55	0.70
≥1 lesion with plaque burden ≥70%	OR 1.12 (0.98-1.29) p=0.093	OR 1.28 (1.05-1.56) p=0.016	OR 1.07 (0.87-1.30) p=0.54	0.21
≥1 lesion with minimal luminal area ≤4.0 mm^2	OR 0.97 (0.86-1.09) p=0.62	OR 0.99 (0.83-1.17) p=0.89	OR 0.99 (0.83-1.19) p=0.92	0.99

Presented results are unadjusted β per standard deviation increase in ln-transformed C-reactive protein or unadjusted odds ratio per standard deviation increase in ln-transformed C-reactive protein with 95% confidence interval.

ACS, acute coronary syndrome; CAD, coronary artery disease; CRP, C-reactive protein; VH-TCFA, virtual histology-derived thin-cap fibroatheroma.

Supplemental table 2. Associations between C-reactive protein and cardiovascular outcome stratified by patients with acute coronary syndrome and patients with stable coronary artery disease

	Total study population	ACS patients	Stable CAD patients	P for heterogeneity
MACE				
CRP 1-3 vs <1 mg/L	HR 1.92 (0.90-4.10) p=0.092	HR 9.10 (1.17-70.5) p=0.035	HR 1.07 (0.42-2.69) p=0.89	0.061
CRP >3 vs <1 mg/L	HR 2.11 (1.02-4.38) p=0.044	HR 6.71 (0.88-51.0) p=0.066	HR 2.15 (0.91-5.10) p=0.083	0.31
Composite of death or ACS				
CRP 1-3 vs <1 mg/L	HR 3.14 (0.86-11.4) p=0.082	HR 5.63 (0.69-45.8) p=0.11	HR 1.60 (0.27-9.59) p=0.61	0.37
CRP >3 vs <1 mg/L	HR 5.13 (1.52-17.3) p=0.009	HR 5.73 (0.74-44.0) p=0.094	HR 5.54 (1.15-26.7) p=0.033	0.98

Presented results are unadjusted hazard ratios with 95% confidence intervals.

ACS, acute coronary syndrome; CAD, coronary artery disease; CRP, C-reactive protein; MACE, major adverse cardiac events.

Supplemental table 3. Association with non-culprit lesion related and indeterminate events only

	Unadjusted HR (95%CI)	P	Adjusted* HR (95%CI)	P
MACE				
CRP 1-3 vs <1 mg/L	1.91 (0.82-4.46)	0.14	1.60 (0.67-3.83)	0.29
CRP >3 vs <1 mg/L	2.12 (0.94-4.78)	0.071	1.91 (0.81-4.49)	0.14
Composite of death or ACS				
CRP 1-3 vs <1 mg/L	2.50 (0.66-9.43)	0.18	1.74 (0.44-6.85)	0.43
CRP >3 vs <1 mg/L	4.58 (1.34-15.6)	0.015	3.40 (0.94-12.3)	0.061

Definite culprit lesion-related events were excluded in the current analyses.

* Adjusted for age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, peripheral artery disease, history of percutaneous coronary intervention, statin use at time of hospital admission and indication for coronary angiography.

ACS, acute coronary syndrome; CRP, C-reactive protein; MACE, major adverse cardiac event.

CORONARY VULNERABILITY

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HIGH-SENSITIVITY TROPONIN T IN RELATION TO CORONARY PLAQUE CHARACTERISTICS IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE; RESULTS OF THE ATHEROREMO-IVUS STUDY

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ABSTRACT

Background and aims: To assess the relationship between the extent and phenotype of coronary atherosclerosis, as assessed by in-vivo grayscale and radiofrequency intravascular ultrasound (IVUS), and circulating Troponin levels in patients with established stable coronary artery disease (CAD).

Methods: In this single-center, cross-sectional analysis, high-sensitivity Troponin T (hsTnT) was measured and IVUS was performed in a predefined non-stenotic segment of a non-culprit coronary artery in 231 patients with stable CAD undergoing elective angiography.

Results: HsTnT was detectable (>3 pg/mL) in 212 patients (92%) and a concentration above 14 pg/mL was observed in 19.5%. Normalised segmental plaque volumes were positively associated with hsTnT levels (25.0 mm^3 increase in segmental plaque volume per SD increase in ln-transformed hsTnT, 95%CI: 6.0-44.0, p=0.010). Higher hsTnT levels were measured in patients with a virtual histology derived thin-cap fibroatheroma (VH-TCFA, adj. odds ratio for presence of VH-TCFA = 1.52 per SD increase in ln-transformed hsTnT, 95%CI: 1.10-2.11, p=0.011). Patients with a VH-TCFA had a 2-fold increased prevalence of hsTnT concentration ≥14 pg/mL (adj. OR 2.35, 95% CI: 1.12-4.91,p=0.024). In addition, a 3-fold increased prevalence of hsTnT concentration ≥14 pg/mL was observed in patients with a VH-TCFA with a lesional plaque volume higher than the median (adj. OR 3.36, 95%CI: 1.44-7.84, p=0.005).

Conclusions: Segmental plaque volume and presence of VH-TCFA lesions are associated with higher circulating hsTnT concentrations in stable CAD patients. Subclinical plaque rupture or erosion and distal embolization may be hypothesized as a potential pathophysiological mechanism with respect to Troponin elevation and its relation with adverse outcome in this patient population.

INTRODUCTION

Cardiac Troponin is the preferred biochemical marker for diagnostic use in patients with a suspected acute coronary syndrome (ACS) [1]. However Troponin elevation also has prognostic relevance in patients *without chestpain at rest*. In ambulatory patients with established stable coronary artery disease (CAD) as enrolled in the Heart and Soul study, TroponinT (TnT) was detectable in 6% of the study population when using a conventional TnT assay [2]. With the recent introduction of the high-sensitivity Troponin T assay (hsTnT), circulating TnT levels could be detected in 81% of the same study population of ambulatory patients with stable CAD [3]. In these 984 patients, higher hsTnT levels were, amongst others, associated with greater inducible ischemia, worse treadmill exercise capacity and lower left ventricular ejection fraction. Moreover, hsTnT elevation remained independently predictive of cardiovascular mortality, myocardial infarction (MI) and heart failure after adjustment for abnormalities in cardiac structure and function [3]. Similar associations between hsTnT elevation and increased risk for major adverse cardiovascular events (MACE) in ambulatory patients with stable CAD were also observed in a post-hoc analyses of the PEACE trial [4], the BARI-2D trial [5] and in a study of stable CAD patients participating in an in-hospital rehabilitation program[6].

Yet, despite these positive associations between hsTnT and long-term outcome, currently no data are available on the association between coronary plaque characteristics and hsTnT elevation in patients with stable CAD. However, the assessment of such a possible relationship is imperative in order to understand the etiology of the Troponin elevation, as well as to gain insight into the mechanisms by which Troponin elevation exerts its adverse impact on prognosis [7]. Hence, our objective was to assess the relationship between coronary plaque characteristics and phenotype, as assessed by in-vivo grayscale and radiofrequency intravascular ultrasound (IVUS), and Troponin levels in patients with established stable CAD.

METHODS

Study population

The design, detailed inclusion and exclusion criteria and initial results of the prospective, single-center, observational, European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study have been described previously [8,9]. The study enrolled 581 patients, but for the current analysis 318 ACS patients were omitted, since hsTnT levels in those patients are obviously more determined by intracoronary thrombosis due to acute plaque rupture of a culprit vessel and subsequent varying degrees of myocardial necrosis, and to a

much lesser extent by the vessel characteristics of a non-culprit vessel, as investigated in the ATHEROREMO-IVUS study. All of the patients had stable angina, were ambulatory and presented at the discretion of the referring physician for a planned and elective admission for angiography after consenting at the out-patient clinic. None of the patients were admitted for acute chest pain or dynamic ECG changes at rest. Patients with an indication for angiography other than stable angina pectoris (SAP, n=9) and those with known confounders of Troponin elevation [10] such as history of heart failure (n=13) or renal insufficiency (n=19, nine of whom had concomitant heart failure) were omitted.

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. ATHEROREMO-IVUS is registered in ClinicalTrials.gov, number NCT01789411.

High-sensitivity Troponin T

Blood samples were drawn from the 6 French arterial sheath prior to catheter insertion. TnT was measured with both a conventional fourth generation assay and a high-sensitivity assay on the Cobas 8000 modular analyzer platform (Roche Diagnostics GmbH, Mannheim, Germany). The diagnostic range of the high-sensitivity assay is 3–10.000 pg/mL with a coefficient of variation of 9% at the 99th percentile value of 14 pg/mL [11]. Laboratory personnel were blinded for baseline patient characteristics and IVUS data.

Intracoronary ultrasound imaging

Subsequent to the standard angiography and PCI (when applicable), IVUS of a non-culprit coronary artery was performed with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA)[9]. The IVUS target segment of the non-culprit coronary artery was required to be at least 40 mm in length and without significant luminal narrowing (< 50% stenosis) as assessed by on-line angiography. Selection of the non-culprit vessel was predefined in the study protocol. The IVUS images were analyzed off-line by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands). The core laboratory personnel were blinded for baseline patient characteristics and TnT and hsTnT levels.

Plaque burden was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area X 100. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. The composition of the atherosclerotic lesions was characterized into 4 different tissue types with the use of IVUS virtual histology (IVUS-VH): fibrous, fibro-fatty, dense calcium and necrotic core. Three types of high-risk lesions were identified: 1. Virtual histology-based thin-cap fibroatheroma (VH-TCFA) lesion, defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen; 2. lesion with large plaque

burden, defined as a lesion with a plaque burden of ≥70%; 3. stenotic lesion, defined as a lesion with a minimal luminal area of ≤4.0 mm^2 [12,13]. VH-TCFAs were further classified as having a high lesional plaque volume in case the plaque volume of that particular VH-TCFA was above the median plaque volume of all lesions classified as VH-TCFA.

Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are presented as median and interquartile range (IQR). Categorical variables are presented in numbers and percentages. Linear regression was used to evaluate the association between segmental plaque volume, plaque burden, plaque tissue types and natural logarithm(ln)-transformed hsTnT concentration (ln-transformation was performed in order to maintain homogeneity of variance and normality of the (error) distribution). Segmental plaque and vessel volume were normalised for the imaged segment length (normalised plaque volume = plaque volume / imaged segment length * median segment length of study population). Logistic regression was used to examine the association between hsTnT concentration and presence of high-risk coronary lesions (as dependent). Determinants of a hsTnT concentration above the clinically used 99th percentile in an apparently healthy reference population of 14 pg/mL [11], were also assessed with logistic regression. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, glomerular filtration rate, hypertension, smoking status, family history of CAD, history of MI, prior PCI, prior coronary artery bypass grafting (CABG), stroke and peripheral artery disease (PAD). Crude and adjusted odds ratios (OR) are presented with 95% confidence intervals. When necessary to assure parsimony of the logistic regression models, adjustment according to a propensity score (using the same confouders as mentioned above) was used [14,15]. All statistical tests were two-sided with a type I error level of 0.05. Analyses were performed with IBM SPSS statistics version 21.0.

RESULTS

Between October 24, 2008 and January 28, 2011, a total of 231 patients with stable angina pectoris were enrolled prior to coronary angiography. Mean age was 63.6 ± 9.9 years. Men constituted 77% of the study population. A PCI was performed in 85% of the patients during the index coronary angiography (Table 1).

Troponin T was detectable in 5.8% of the study patients by the conventional TnT assay (>0.01 ug/L). In contrast, the hsTnT assay enabled detection (>3 pg/mL) in 212 patients (92%) and concentrations above the commonly used 99th percentile of a healthy reference population of 14 pg/mL were observed in 45 (19.5%) of our patients with manifest

Table 1. **Baseline characteristics**	
Patient characteristics, N (%)	**N=231**
Age (years±SD)	63.6 ± 9.9
Male	177 (76.6)
Diabetes Mellitus	48 (20.8)
Hypertension	141 (61.0)
Hypercholesterolemia	162 (70.1)
Smoking	42 (18.2)
Positive family history of CAD	137 (59.3)
Previous MI	93 (40.3)
Previous PCI	116 (50.2)
Previous CABG	7 (3.0)
Previous stroke	13 (5.6)
Peripheral artery disease	19 (8.2)
Glomerular Filtration Rate (ml/min, median[IQR])	101.5 [80.2 – 123.0]
Out-patient clinic medication prior to angiography	
Aspirin	218 (94.4)
Beta-blockers	181 (78.4)
ACE-inhibitors	139 (60.2)
Calcium antagonists	63 (27.3)
Oral nitrates	82 (35.5)
Statin	209 (90.5)
High-sensitivity Troponin T levels (pg/mL)	
Median [IQR]	7.3 [4.9 - 12.1]
Mean	11.0
Standard deviation	15.5
Range	3.00 – 192.70
99th percentile	88.7
Procedural characteristics	
Extent of coronary artery disease	
No significant stenosis	22 (9.5)
1-vessel disease	116 (50.2)
2-vessel disease	70 (30.3)
3-vessel disease	23 (10.0)
PCI / stent implantation	196 (84.8)

Table 1 *(continued)*	
IVUS segment characteristics	
Imaged coronary artery	
Left anterior descending	76 (33.9)
Left circumflex	76 (32.9)
Right coronary artery	79 (34.2)
Segment length, mm [IQR]	43.8 [33.9 - 56.0]

MI= myocardial infarction; PCI= percutaneous coronary intervention; CABG= coronary artery bypass graft

stable CAD. The 99th percentile in our patient population was 88.7 pg/mL (Table 1 and Figure 1).

Clinical determinants of high-sensitivity Troponin T concentration

Age was the only determinant of hsTnT concentration (adjusted (adj.) p<0.001) (Figure 2). There was no association with hsTnT concentration and other baseline clinical variables, such as male gender (adj. p=0.39), hypercholesterolemia (adj. p=0.28), diabetes (adj. p=0.96), glomerular filtration rate (adj. p=0.08), hypertension (adj. p=0.64), smoking status (adj. p=0.37), family history (adj. p=0.60), history of MI (adj. p=0.57), PCI (adj. p=0.60), CABG (adj. p=0.46), stroke (adj. p=0.61), or PAD (adj. p=0.28), and the number of diseased coronary vessels on angiography (adj. p=0.71).

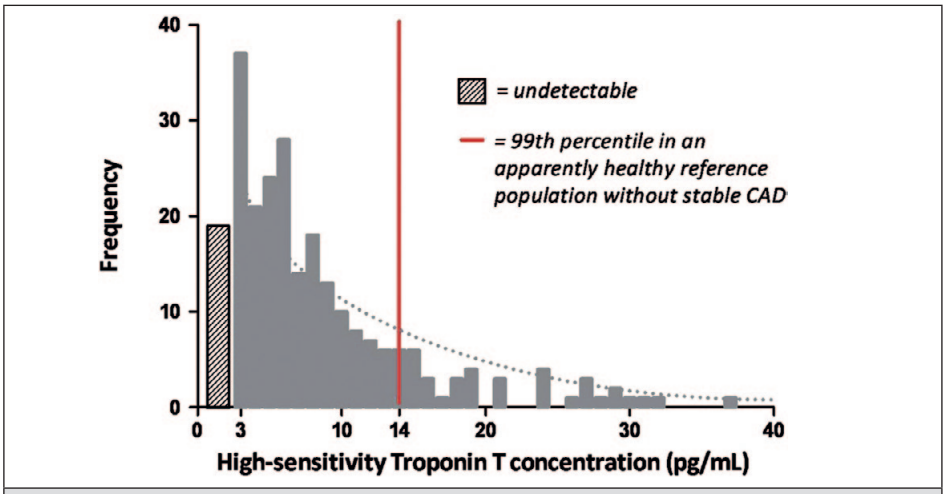

Figure 1. Distribution of high-sensitivity Troponin T values in patients with stable coronary artery disease.
High-sensitivity Troponin T was measured in 231 patients with stable coronary artery disease, undergoing an elective CAG. Blood samples were drawn prior to catheterisation and/or PCI. Troponin concentrations were undetectably low in 19 patients (8.2%). The datapoints of three patients with concentrations of 82, 91 and 192 pg/ml respectively are not shown in this histogram.

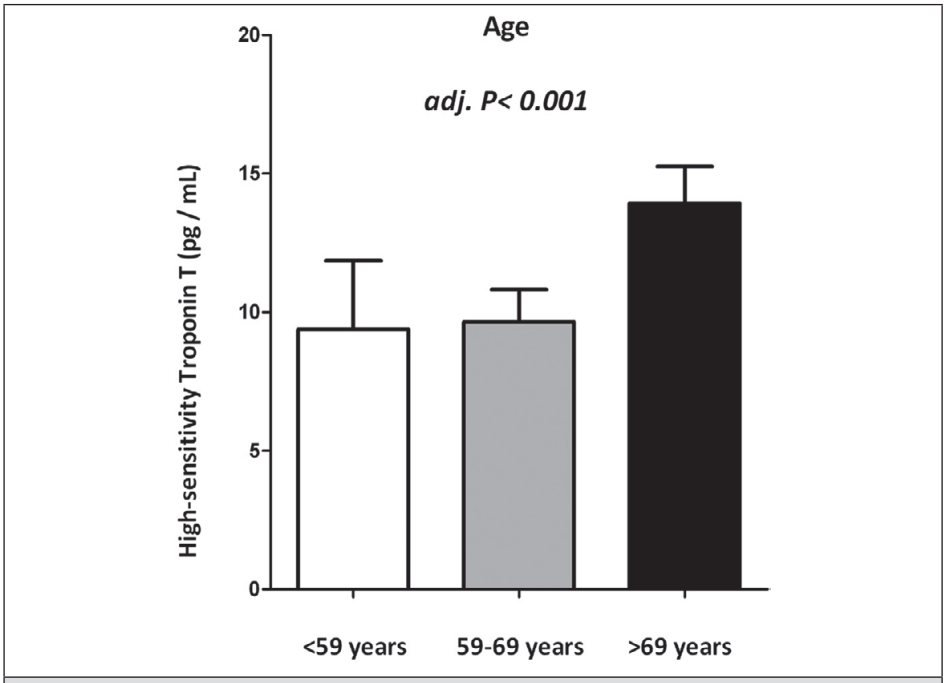

Figure 2. Age in relation to high-sensitivity Troponin T concentration.
The study population was divided in age tertiles of 77 patients each. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, glomerular filtration rate, hypertension, smoking status, family history of CAD, history of MI, prior PCI, prior coronary artery bypass grafting, prior stroke and peripheral artery disease. The histograms display the mean hsTnT concentration plus the standard error of the mean.

High-sensitivity Troponin T in association with segmental plaque characteristics.

The median segment length, as imaged with IVUS, was 43.8 mm (Table1). Normalised segmental plaque volumes were positively associated with hsTnT levels (adjusted β= 25.0 mm^3 increase in segmental plaque volume per SD increase in natural log-transformed hsTnT, 95% CI: 6.0-44.0, p=0.010) (Table 2, Figure 3 A). There was no significant association between segmental plaque burden and hsTnT (adjusted β= 0.92% increase in plaque burden per SD increase in natural log-transformed hsTnT, 95% CI: −0.61-2.44, p=0.24). However normalised segmental vessel volumes were positively associated with hsTnT levels (adjusted β= 56.2 mm^3 increase in segmental vessel volume per SD increase in natural log-transformed hsTnT, 95% CI: 21.9-90.5, p=0.001).

High-sensitivity Troponin T concentrations were not associated with the segmental plaque distribution of the four tissue types as assessed by IVUS virtual histology; fibrous (adj. p=0.81), fibro-fatty (adj. p=0.66), dense calcium (adj. p=0.37) and necrotic core (adj. p=0.80).

Table 2. High-sensitivity Troponin T concentration in relation to plaque characteristics in a non-culprit coronary artery in patients with stable coronary artery disease

	Total study population	hsTnT < 14 pg/ml (N= 186)	hsTnT ≥ 14 pg/ml (N=45)	Adjusted P-value
SEGMENTAL PLAQUE CHARACTERISTICS	***Median [IQR]***			
Normalised vessel volume (mm^3)	563.9 [439.2 – 755.0]	545.5 [421.2 – 691.3]	733.6 [494.8 - 917.5]	0.001
Normalised plaque volume (mm^3)	234.0 [149.9 - 340.6]	215.2 [140.8 - 311.2]	271.1 [192.4 - 413.7]	0.008
Plaque burden (%)	40.4 [32.2 - 47.7]	39.8 [31.9 - 47.1]	43.2 [33.8 - 50.2]	0.41
Plaque composition	***Median [IQR]***			
Fibrous (%)	56.3 [49.4 - 63.8]	56.0 [49.5 - 65.1]	56.6 [48.6 – 60.3]	0.49
Fibro-fatty (%)	9.5 [6.3 - 13.4]	9.3 [5.8 - 13.4]	11.2 [8.1 - 14.2]	0.18
Dense calcium (%)	11.0 [5.9 - 16.1]	10.9 [5.7 - 16.1]	11.4 [6.3 - 17.1]	0.79
Necrotic core (%)	21.5 [17.2 - 25.3]	21.5 [16.7 - 25.7]	21.5 [18.4 - 24.9]	0.82
LESION MORPHOLOGY	***N (%)***			
VH-TCFA	86 (37.2)	64 (34.4)	22 (48.9)	0.024
VH-TCFA with high lesional plaque volume	43 (18.6)	28 (15.1)	15 (33.3)	0.005
MLA ≤ 4.0 mm^2	80 (34.6)	67 (36.0)	13 (28.9)	0.66
Plaque burden ≥ 70%	56 (24.2)	41 (22.0)	15 (33.3)	0.064

VH-TCFAs were classified as having a high lesional plaque volume in case the plaque volume of that particular VH-TCFA was above the median of all lesions classified as VH-TCFA. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, glomerular filtration rate, hypertension, smoking status, family history of CAD, history of MI, prior PCI, coronary artery bypass grafting, stroke and peripheral artery disease (PAD).

HsTnT= high-sensitivity Troponin T; VH-TCFA= Virtual histology-derived Thin-cap fibroatheroma; MLA= minimal luminal area

High-sensitivity Troponin T in association with lesion characteristics and morphology.

With respect to lesion morphology, a VH-TCFA was observed in 86 (37%) patients (Table 2 and Supplementary appendix Figure 4). Higher hsTnT levels were measured in patients with the presence of a VH-TCFA (adj. OR for presence of VH-TCFA= 1.52 per SD increase in natural log-transformed hsTnT, 95% CI: 1.10-2.11, p=0.011) (Figure 3B).

In patients with hsTnT concentrations ≥ 14 pg/mL, a VH-TCFA was observed in 49%. Hence, patients with a VH-TCFA had a 2-fold increased prevalence of hsTnT concentration ≥ 14 pg/mL (adj. OR 2.35, 95% CI: 1.12-4.91, p=0.024) (Table2). In addition, a 3-fold increased prevalence of hsTnT concentration ≥ 14 pg/mL was observed in patients with a VH-TCFA with a high lesional plaque volume, i.e. a lesional plaque volume above the

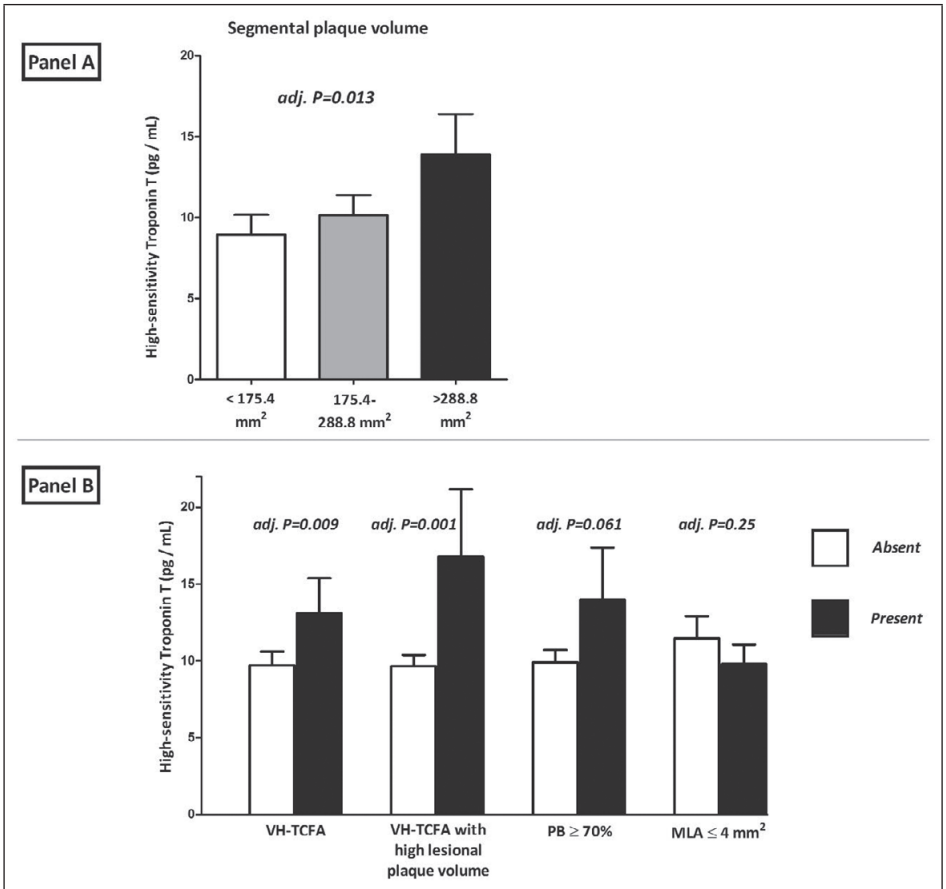

Figure 3. High-sensitivity Troponin T concentration in relation to intravascular ultrasound characteristics.

HsTnT, high-sensitivity Troponin T; PB, plaque burden; MLA, minimal luminal area, VH-TCFA, Virtual histology-derived thin-cap fibroatheroma.

Panel A. High-sensitivity Troponin T per tertile of normalised plaque volume for the entire segment (median length 43.8 mm, IQR: 33.9 - 56.0) as evaluated with grayscale intravascular ultrasonography (IVUS).

Panel B. High-sensitivity Troponin T in relation to the presence of at least one high-risk lesion type per patient. Thin-cap fibroatheroma (TCFA) was assessed by means of radiofrequency IVUS.VH-TCFAs were classified as having a high lesional plaque volume in case the plaque volume of that particular VH-TCFA was above the median plaque volume of all lesions classified as VH-TCFA.

For both figures, multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, glomerular filtration rate, hypertension, smoking status, family history of CAD, history of MI, prior PCI, coronary artery bypass grafting, stroke and peripheral artery disease. The histograms display the mean hsTnT concentration plus the standard error of the mean.

median of all lesions classified as VH-TCFA (adj. OR 3.36, 95% CI: 1.44-7.84, p=0.005) (Table 2). Patients with a VH-TCFA with a high lesional plaque volume also had higher hsTnT levels than patients with a VH-TCFA without a high lesional plaque volume (16.8 pg/mL versus 9.4 pg/mL, p=0.03).

The relationship between lesions with a plaque burden ≥ 70% and hsTnT was not statistically significant (adj. OR= 1.37 per SD increase in natural log-transformed hsTnT, 95% CI: 0.99-1.90, p=0.059) (Table 2 and Figure3B). No association was found between stenotic lesions with a MLA ≤ 4.0 mm^2 and hsTnT (adj. OR= 0.83 per SD increase in natural log-transformed hsTnT, 95% CI: 0.60-1.15, p=0.26).

DISCUSSION

This cross-sectional study is the first to demonstrate an association between elevated circulating Troponin levels and the extent of coronary atherosclerosis and high-risk plaque phenotype, as assessed with intracoronary IVUS in a non-culprit coronary artery in a broad population of stable CAD patients referred for elective coronary angiography, as seen in everyday routine clinical practice.

In contrast to the conventional TnT assay, the hsTnT assay has enabled detection of circulating TnT in the majority of patients with stable CAD. Our finding that serum TnT levels were detectable in only 5.8% of our stable CAD patients with the conventional fourth generation TnT assay, versus detection in 92% when using the hsTnT assay, corresponds to the results found in the Heart and Soul study [3]. This expansion of serum TnT detection has led to more elaborate risk prediction for the occurrence of MACE, as previously demonstrated [3,4,6,16]. In a post-hoc analysis of the relatively low-risk, stable CAD population of the PEACE trial, a graded increase in the cumulative incidence of cardiovascular death (adjusted hazard ratio (HR) per unit increase in the natural logarithm of the hsTnT level 2.09; 95% confidence interval [CI], 1.60 to 2.74) and of heart failure (adjusted HR 2.20; 95% CI, 1.66 to 2.90) was seen in 3679 patients with stable CAD and preserved left ventricular function [4]. Similarly hsTnT was an independent predictor of death from cardiovascular causes, myocardial infarction, or stroke in patients who had both type 2 diabetes and stable ischemic heart disease in a post-hoc analysis of the BARI-2D trial [5]. In addition, several prospective, intracoronary imaging studies, primarily conducted in ACS-patients, have reported an increased risk of repeat MACE in the presence of TCFA as identified by IVUS-VH [17,18]. The presence of a VH-TCFA was associated with a 3-fold increased risk of cardiovascular mortality, cardiac arrest, MI, or rehospitalization due to unstable or progressive angina during 3.4 year follow-up of ACS patients enrolled in the PROSPECT study [17]. More recently, similar conclusions were drawn with respect to the complete ATHEROREMO-IVUS study population comprising

both stable CAD and ACS patients [9]. The presence of a VH-TCFA, in a single target segment without significant luminal narrowing in a non-culprit coronary artery, was independently associated with the composite of death and non-fatal ACS (7.5% vs. 3.0%; adjusted HR 2.51, 95% CI 1.15-5.49) during 1-year follow-up [9].

Thus, both TnT elevation and high-risk intracoronary lesion phenotypes as assessed by radiofrequency IVUS are recognized as independent predictors of adverse cardiovascular outcome. Yet, to our best knowledge, this is the first study to describe the crosslink between TnT elevation and the extent and phenotype of coronary atherosclerosis as assessed by IVUS in a non-culprit coronary artery in a population of patients with stable CAD.

Despite the increasing body of evidence showing adverse outcome in case of Troponin elevation in ambulatory non-ACS patients, only few reports have actually provided insight into a possible mechanistic explanation for the Troponin elevation from the perspective of coronary pathophysiology. A greater insight into the pathophysiological mechanisms of Troponin elevation may also increase our understanding of how Troponin elevation is linked to adverse outcome in this patient population. In two studies evaluating cardiac computed tomography, one in patients with stable CAD and another in patients with acute chest pain presenting at the emergency department, hsTnT levels were not associated with stenosis severity, but were associated with the extent of coronary plaque volume, which might support the hypothesis that chronic, clinically silent rupture or erosion of non-calcified plaques with subsequent microembolisation may be a potential source of myocardial injury and Troponin release [19,20]. However, given the current state-of-the-art, cardiac computed tomography does not allow for such extensive plaque phenotyping as grayscale and radiofrequency IVUS. Therefore, our data showing that VH-TCFAs are associated with Troponin release are essential for the line of reasoning and hypothesis that microembolisation resulting from silent plaque rupture or erosion might be a possible mechanistic explanation for the elevated Troponin levels in these patients. Indeed, autopsy studies have described TCFAs to be the plaques that are most prone to superficial erosion or rupture, consequently leading to thrombus formation and distal (micro)embolisation [21]. Such plaque erosion or rupture has shown to be the major cause of (fatal) acute MI, but not every plaque erosion or rupture invariably leads to sufficient thrombotic occlusion in order to provoke symptoms of angina. It has been suggested that, at any given time, approximately 15% of patients with stable CAD have ongoing atherothrombotic plaque events compared to an annual incidence of acute MI of approximately 5% [22]. Against this background, hsTnT may serve as a biomarker for subclinical plaque rupture or erosion leading to atherothrombosis, distal embolisation and continuous low grade myocardial ischemia and cardiomyocyte necrosis, even in presumably asymptomatic patients with stable CAD.

Similarly, the association between the presence of VH-TCFA and serum TnT elevation as found in the current population might also be extended in order to hypothesize on the observation of subtle TnT elevation in the general population and the associated increased risk of MACE. In the Dallas Heart Study, a multi-ethnic, population-based cohort study of individuals aged 30 to 65 years, the prevalence of TnT levels above 3.0 pg/mL was 25.0% [16]. Interestingly, a graded increase in both cardiovascular and all-cause mortality was seen across quintiles of TnT elevation in the entire study cohort, but also in the subgroup analysis of 3222 patients without cardiovascular or chronic kidney disease. A five-fold increased risk for cardiovascular mortality was observed in case of hsTnT levels ≥ 14 pg/mL. Understandably, the Dallas Heart study did not collect intracoronary IVUS data. Although speculative, an increased prevalence of rupture-prone, high-risk lesion types, such as TCFAs, might underlie the observation that participants without symptoms of angina, i.e. without a fixed and significant coronary luminal narrowing, did have both TnT elevation and a subsequent increased risk of cardiovascular mortality.

Another important finding of our study was the association between segmental plaque volume and Troponin concentration. Similar observations were previously found in cardiac computed tomography studies [19,20]. More recently, a post-hoc analysis of the SATURN trial has demonstrated that a large plaque volume as assessed with grayscale IVUS, in a non-culprit segment without significant stenosis, is associated with increased risk of MACE [23]. Hence, segmental plaque volume of a non-culprit coronary artery, as assessed in ATHEROREMO-IVUS, may have prognostic importance. Segmental plaque volume may not only be linked to Troponin concentration, but also to TCFA and plaque rupture, since the majority of large stable plaques have evidence of previously healed plaque rupture with incorporation of old thrombus into the atheroma [21,22,24,25].

Troponin was not related to segmental plaque burden (p=0.24). Levels seemed higher in patients with a lesion with plaque burden ≥ 70%, but the difference with patients with smaller plaque burden did not reach statistical significance (p=0.061). This may be due to the fact that plaque burden is not a direct measure of three dimensional plaque volume, but rather a two dimensional measure that also accounts for arterial wall remodeling. The fact that both normalized plaque and vessel volume were highly associated with troponin levels may be seen as a confirmation of positive remodeling in our dataset. Outward remodeling explains why presence of large segmental plaque volumes do not necessarily relate to focal lesional stenoses. The observation that stenosis severity, i.e. a MLA ≤ 4.0 mm^2, was not related to hsTnT concentration in our study may be regarded as a reconfirmation of the earlier mentioned cardiac computed tomography studies [19,20,26]. Similarly, coronary artery calciumscore was not associated with hsTnT concentration after multivariate adjustment in the in the Dallas Heart Study [16]. Such observations may indirectly support the hypothesis that not ischemia due to luminal narrowing, but rather plaque rupture, microembolisation and microcirculatory

dysfunction is the pathophysiological mechanism behind the increased circulating TnT levels [27]. On the other hand, it has to be emphasized that 84.8% of the patients in our analysis underwent a PCI and therefore had significant luminal stenosis in the culprit vessel. Our protocol was based on non-culprit, single vessel imaging. Since the culprit vessels were not imaged, all of our associations only apply to the angiographically non-stenotic non-culprit segments and no formal conclusions can be drawn on stenosis severity elsewhere in the coronary circulation and Troponin elevation.

Our study has several strengths. Our data were prospectively obtained and, due to the broad inclusion criteria of ATHEROREMO-IVUS, its conclusions seem applicable to a broad range of patients with stable CAD. Of great importance is that IVUS evaluation was performed in an independent, dedicated core lab with personnel blinded for patient and TnT data. Similarly, Troponin was measured by laboratory personnel blinded for baseline patient characteristics and IVUS data.

Study limitations

However, there are several limitations to our findings. A possible limitation of our analysis might be the sample size, although this study represents the only and therefore largest cohort of patients in which the association between intracoronary IVUS plaque characteristics and circulating hsTnT was evaluated, so far. Furthermore, ATHEROREMO-IVUS was a single center study by virtue of design. External validation, preferably in a larger sample size, is a fundamental prerequisite before final conclusions may be drawn. In our study, IVUS imaging took place of a pre-specified single target segment of a non-culprit coronary artery of least 40 mm in length and without significant luminal narrowing (< 50% stenosis) as assessed by on-line angiography. This approach was developed under the assumption that such a non-stenotic segment would adequately reflect coronary wall pathophysiology of the larger coronary tree. Indeed, in a previous ATHEROREMO-IVUS report, this assumption was confirmed with respect to the presence of high-risk lesion types, such as VH-TCFA, and subsequent increased risk of MACE [9]. In addition, the post-hoc analysis of the SATURN trial also emphasized the prognostic importance of plaque characteristics of a non-stenotic, non-culprit target segment [23]. Ideally, a confirmatory replication of our association between segmental plaque volume, presence of (VH-)TCFA and TnT elevation should take place in a study enrolling patients with stable CAD for three-vessel and left main IVUS assessment, since such an approach would more precisely characterize the total coronary atherosclerosis burden. IVUS-VH has been validated *in vitro* [28]. Sensitivities and specificities for the detection of various plaque components ranged from 72-99%, thus leaving room for misclassification [28]. Furthermore, its ability to detect plaque erosion, rupture and thrombus is limited given the current spatial resolution.

In conclusion, plaque volume and presence of VH-TCFAs, as assessed with intracoronary IVUS in a non-culprit coronary artery segment, are associated with higher circulating Troponin concentrations in a broad population of patients with stable CAD. Our data are based on associations and cannot provide final conclusions on the exact etiology of Troponin elevation. However our findings may generate the hypothesis that subclinical plaque rupture or erosion of vulnerable plaques and subsequent intracoronary thrombosis and distal embolization may be the potential mechanism of action with respect to Troponin elevation and its relation with adverse outcome.

Funding

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Conflict of interest

None. There is no commercial association that might pose a conflict of interest in connection with this manuscript.

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ONLINE SUPPLEMENT

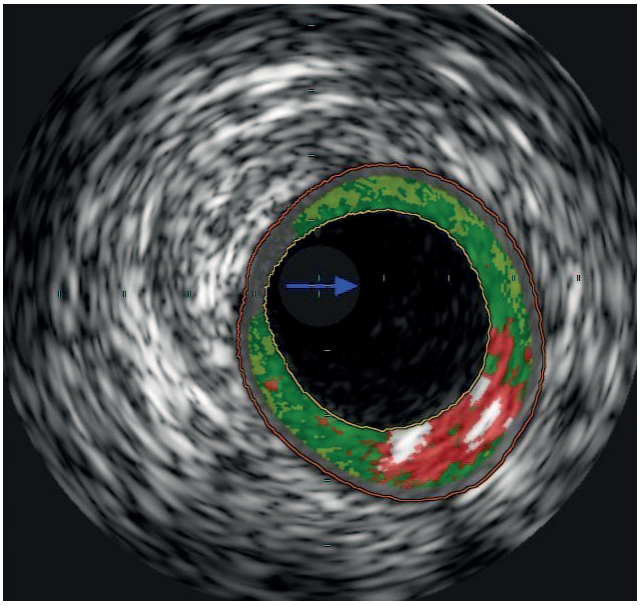

Figure 4. Thin-cap fibroatheroma as classified by radiofrequency intravascular ultrasonography
This lesion (here only represented by one frame) in the proximal right coronary artery of a 76-year old ATHEROREMO-patient was classified as virtual histology thin-cap fibroatheroma. It demonstrated a large plaque volume (298 mm^3) and plaque burden(61%), nevertheless without luminal narrowing (luminal area of 9.0 mm^2) The high-sensitivity Troponin T concentration in this patient was 14 pg/mL.

CORONARY VULNERABILITY

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PCSK9 IN RELATION TO CORONARY PLAQUE INFLAMMATION: RESULTS OF THE ATHEROREMO-IVUS STUDY

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ABSTRACT

Background and aims: Experimental studies have suggested that proprotein convertase substilisin/kexin type 9 (PCSK9) might directly promote inflammatory processes contributing to atherosclerosis by mechanisms independent of low-density lipoprotein (LDL) cholesterol levels. This study aims to investigate the association between serum PCSK9 levels and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by intravascular ultrasound virtual histology (IVUS-VH) imaging.

Methods: Between 2008 and 2011, IVUS-VH imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for acute coronary syndrome (ACS) or stable angina. PCSK9 concentrations were measured in serum samples that were drawn prior to coronary angiography. None of the patients received PCSK9 inhibitors.

Results: After adjustment for established cardiac risk factors, statin use and serum LDL cholesterol, serum PCSK9 levels were linearly associated with the fraction of plaque consisting of necrotic core tissue (β=1.24 percent increase per 100μg/L increase in PCKS9, 95%CI 0.55-1.94, p=0.001) and with the absolute volume of necrotic core tissue (β=0.09, 95%CI 0.01-0.18, p=0.033), but were not significantly associated with plaque burden (p=0.11), plaque volume (p=0.22) or the presence of IVUS-VH-derived thin-cap fibroatheroma lesions (p=1.0).

Conclusion: Serum PCSK9 levels were linearly associated with the fraction and amount of necrotic core tissue in coronary atherosclerosis, independently of serum LDL cholesterol levels and statin use. Therefore, PCSK9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL cholesterol regulation.

Key words: PCSK9, atherosclerosis, inflammation, intravascular ultrasound, prognosis

INTRODUCTION

Proprotein convertase substilisin/kexin type 9 (PCSK9) has an important role in the degradation of low-density lipoprotein (LDL) receptors, resulting in increased serum LDL cholesterol concentrations.[1,2] Novel drugs targeting PCSK9 are currently being investigated in large phase II and phase III clinical trials.[2-9] Most of these trials investigate the effects of PCSK9 inhibition on LDL cholesterol reduction. However, recent experimental studies have also demonstrated that PCSK9 might directly promote inflammation, apoptotic cell death and endothelial dysfunction in atherosclerosis by mechanisms that are independent of its effect on the LDL receptor.[2,10-12] Therefore, it has been hypothesized that PCSK9 contributes directly to the progression of atherosclerotic disease, beyond its indirect role in cholesterol homeostasis.[2]

Intravascular ultrasound virtual histology (IVUS-VH) is an in-vivo imaging technique that analyzes radiofrequency backscatter.[13] IVUS-VH imaging allows for accurate measurement of the extent of coronary atherosclerosis and of the type of plaque tissue, including necrotic core tissue which is considered to be a result of continuous inflammation.[13-17] Previous studies have demonstrated that the amount of necrotic core tissue on IVUS-VH is predictive of cardiovascular outcome.[14-16] This study aims to investigate the association between serum PCSK9 levels and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS-VH imaging.

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 in detail elsewhere (ClinicalTrials.gov NCT01789411).[14,18] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris were included. None of the patients were treated with drugs targeting PCSK9 during the study period. This study was approved by the medical ethics committee of Erasmus MC. Written informed consent was obtained from all included patients.

Serum proprotein convertase substilisin/kexin type 9

Blood samples were drawn from the arterial sheath prior to coronary angiography, and were stored at a temperature of −80°C within 2 hours after blood collection. PCSK9 concentrations were measured in the stored serum samples (n=576) using an enzyme-linked immunosorbent assay (Human PCSK9 Quantikine ELISA, R&D Systems Inc., Min-

neapolis, MN, USA). The minimum detectable concentration of this assay was 0.096 µg/L with a coefficient of variation of 4.1% at a mean value of 27.9 µg/L. In 5 patients, serum samples were not available for PCSK9 measurement.

Coronary intravascular ultrasound imaging

Following the standard coronary angiography procedure, IVUS-VH imaging of the most proximal part of a non-culprit coronary artery was performed. Offline analysis of the IVUS-VH images was performed by an independent core laboratory (Cardialysis bv, Rotterdam, the Netherlands) that was blinded for patient characteristics, PCSK9 levels and clinical outcome data. Extent and phenotype of the atherosclerotic plaque were assessed (Figure 1). Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. Plaque volume was adjusted for the imaged segment length (adjusted plaque volume = plaque volume / imaged segment length * median segment length in study population). The composition of atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core (Figure 1).[13] The fraction of coronary atherosclerosis consisting of necrotic core tissue was a priori defined as primary outcome measure. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. An IVUS-VH-derived TCFA lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen in at least three consecutive frames.[14-16,18-20]

Statistical analysis

The distributions of the continuous variables, including PCSK9 levels and the IVUS-VH parameters, were assessed for normality by visual examination of the histogram. The non-normally distributed variables (i.e. plaque volume and necrotic core volume) were root-transformed. Linear regression analyses were performed to evaluate the associations of serum PCSK9 levels with 1. plaque burden; 2. plaque volume; 3. fraction of plaque consisting of necrotic core tissue; and 4. necrotic core volume. The results are presented as β with 95% confidence interval (95% CI). Logistic regression analyses were performed to evaluate the association between serum PCSK9 levels and the presence of IVUS-VH-derived TCFA lesions. The results are presented as odds ratios (OR) with 95% CI. First, all analyses were performed univariably. In subsequent multivariate analyses, the variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, indication for coronary angiography (ACS or stable CAD) and statin use at time of hospital admission were a priori defined as potential confounders. Hereafter, baseline serum LDL cholesterol levels was additionally entered into the model to evaluate whether the associations between PCSK9 and coronary plaque characteristics were independent of serum LDL cholesterol levels. Additionally, stratified analyses were performed to evaluate

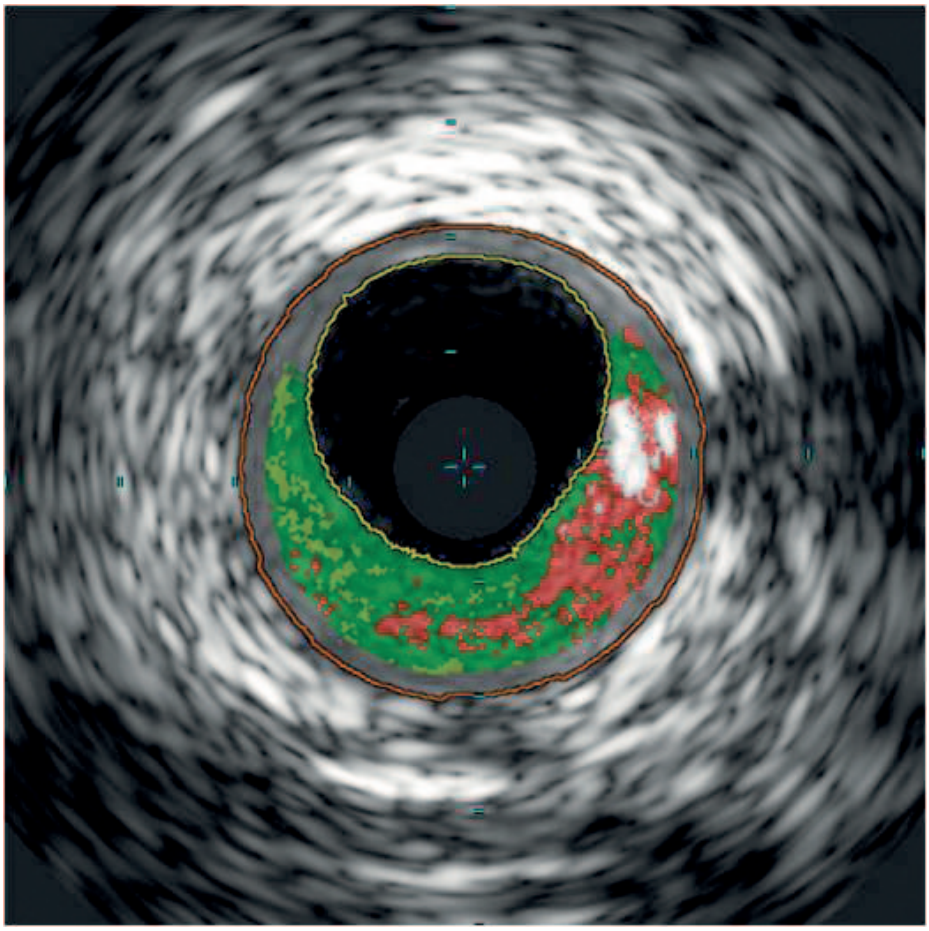

Figure 1. Intravascular ultrasound virtual histology imaging
Intravascular ultrasound virtual histology imaging was used to characterize atherosclerotic plaques into 4 different tissue types: fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red).

whether the observed associations between PCSK9 and coronary plaque characteristics in the total study population were applicable for all patient subgroups (including statin users and non-statin users, as well as patients with low and high LDL cholesterol). 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

Patient characteristics

The patient characteristics are described in Table 1. Median serum PCSK9 level was 270 µg/L and ranged from 91 to 804 µg/L [interquartile range 217-336]. PCSK9 levels were higher in patients with hypertension (median 283 [227-347] versus 255 [215-335] µg/L, p=0.005), hypercholesterolemia (median 281 [228-350} versus 255 [210-328] µg/L, p=0.001), and statin use at time of hospital admission (median 280 [221-342] versus 253 [208-323] µg/L, p=0004). PCSK9 levels did not differ between patients admitted with ACS and patients with stable CAD (p=0.19). The median length of the imaged coronary segment was 44.3 [33.8-55.5] mm. Mean plaque burden in the imaged coronary segment was 38.2 ± 11.5 percent and median plaque volume was 222 [147-326] mm^3. Mean fraction of plaque that consisted of necrotic core was 21.4 ± 8.0 percent and median necrotic core volume was 21.1 [8.6-41.6] mm^3. A total of 241 (42%) patients had at least one IVUS-VH-derived TCFA lesion.

Association between PCSK9 level and coronary plaque characteristics

In univariate analysis, higher serum PCSK9 levels were linearly associated with a higher necrotic core fraction (β = 1.31 percent increase in necrotic core per 100 µg/L increase

Table 1. Baseline characteristics

	n = 576 patients
Patient characteristics	
Age, years	61.5 ± 11.3
Men, n (%)	435 (75.5)
Diabetes Mellitus, n (%)	99 (17.2)
Hypertension, n (%)	300 (52.1)
Hypercholesterolemia, n (%)	320 (55.6)
Current smoking, n (%)	167 (29.0)
Positive family history, n (%)	300 (52.1)
Previous MI, n (%)	183 (31.8)
Previous PCI, n (%)	186 (32.3)
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 (5.6)
History of heart failure, n (%)	19 (3.3)
Serum LDL cholesterol, mmol/L	2.72 [2.12-3.54]

Table 1. *(continued)*	
Serum PCSK9, µg/L	270 [217-336]
Statin use at time of hospital admission, n (%)	359 (62.3)
Procedural characteristics	
Indication for coronary angiography	
ACS, n (%)	314 (54.5)
ST-elevation MI	164 (28.5)
Non-ST-elevation ACS	150 (26.0)
Stable coronary artery disease, n (%)	262 (45.5)
Number of diseased coronary vessels *	
No significant stenosis, n (%)	42 (7.3)
1-vessel disease, n (%)	306 (53.1)
2-vessel disease, n (%)	167 (29.0)
3-vessel disease, n (%)	61 (10.6)
PCI performed, n (%)	507 (88.0)
IVUS-VH imaging	
Imaged coronary artery	
Left anterior descending, n (%)	207 (35.9)
Left circumflex, n (%)	193 (33.5)
Right coronary artery, n (%)	176 (30.6)
Segment length, mm	44.3 [33.8-55.5]
Plaque burden, %	38.2 ± 11.5
Plaque volume †, mm^3	222 [147-326]
Fibrous tissue fraction, %	57.8 ± 11.6
Fibro-fatty tissue fraction, %	8.9 [5.7-12.6]
Dense calcium fraction, %	9.3 [5.1-15.1]
Necrotic core fraction, %	21.4 ± 8.0
Fibrous tissue volume †, mm^3	56.2 [26.9-95.9]
Fibro-fatty volume †, mm^3	7.7 [3.4-17.2]
Dense calcium volume †, mm^3	8.9 [2.9-20.7]
Necrotic core volume †, mm^3	21.1 [8.6-41.6]
≥1 IVUS-VH-derived TCFA lesion, n (%)	241 (41.8)

Data are presented as mean ± standard deviation or as median [interquartile range].

* A significant stenosis was defined as a stenosis ≥50% of vessel diameter by visual assessment on the coronary angiogram.

† Adjusted for imaged segment length.

ACS = acute coronary syndrome; CABG = coronary artery bypass grafting; IVUS-VH = intravascular ultrasound virtual histology; LDL = low-density lipoprotein; MI = myocardial infarction; PCI = percutaneous coronary intervention; PCSK9 = proprotein convertase substilisin/kexin type 9; TCFA = thin-cap fibroatheroma.

Table 2. Association between serum PCSK9 level and coronary plaque characteristics

	Unadjusted	P-value	Adjusted for cardiac risk factors + ACS or stable CAD + statin use *	P-value	Adjusted for cardiac risk factors + ACS or stable CAD + statin use + serum LDL *	P-value
Plaque burden †	β 0.62 (−0.36;1.59)	0.22	β 0.77 (−0.19;1.73)	0.12	β 0.78 (−0.19;1.74)	0.11
Plaque volume ‡	β 0.03 (−0.06;0.12)	0.48	β 0.05 (−0.03;0.14)	0.22	β 0.05 (−0.03;0.14)	0.22
Necrotic core fraction §	β 1.31 (0.63;1.99)	<0.001	β 1.22 (0.52;1.91)	0.001	β 1.24 (0.55;1.94)	0.001
Necrotic core volume ||	β 0.08 (−0.01;0.16)	0.075	β 0.09 (0.01;0.18)	0.036	β 0.09 (0.01;0.18)	0.033
≥1 IVUS-VH-derived TCFA lesion	OR 1.01 (0.85-1.20)	0.93	OR 1.00 (0.84-1.20)	0.99	OR 1.00 (0.84-1.20)	1.0

* Cardiac risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking. Statin use was registered at the time of hospital admission.
† β (95% confidence interval) is increase in plaque burden (%) per 100 µg/L increase in PCKS9.
‡ β (95% confidence interval) is increase in standard deviations of square root transformed plaque volume (mm^3) per 100 µg/L increase in PCKS9. Plaque volume is adjusted for imaged segment length.
§ β (95% confidence interval) is increase in necrotic core fraction (%) per 100 µg/L increase in PCKS9.
|| β (95% confidence interval) is increase in standard deviations of square root transformed necrotic core volume (mm^3) per 100 µg/L increase in PCKS9. Necrotic core volume is adjusted for imaged segment length.
ACS = acute coronary syndrome; CAD = coronary artery disease; IVUS-VH = intravascular ultrasound virtual histology; LDL = low-density lipoprotein; PCSK9 = proprotein convertase substilisin/kexin type 9; TCFA = thin-cap fibroatheroma.

in PCSK9, 95% CI 0.63;1.99, p<0.001) and tended to be associated with a higher absolute necrotic core volume (β = 0.08, 95%CI −0.01;0.16, p=0.075) (Table 2 and Figure 2). Furthermore, PCSK9 levels were inversely associated with fractions of fibrous tissue (β = −1.45, 95%CI −2.43;−0.47, p=0.004) and fibro-fatty tissue (β = −0.83, 95%CI −1.36;−0.30, p=0.002), and positively associated with dense calcium fraction (β 0.97, 95%CI 0.32;1.62, p=0.004) (Supplemental Figure 1). PCSK9 levels were not associated with overall plaque burden (β = 0.62, 95%CI −0.36;1.59, p=0.22), plaque volume (β = 0.03, 95%CI −0.06;0.12, p=0.48) or the presence of IVUS-VH-derived TCFA lesions (OR 1.01, 95%CI 0.85-1.20, p=0.93). After adjustment in multivariate analysis, PCSK9 levels remained significantly associated with both necrotic core fraction (β = 1.22 percent increase in necrotic core per 100 µg/L increase in PCSK9, 95% CI 0.52-1.91, p<0.001) and absolute necrotic core volume (β = 0.09, 95%CI 0.01;0.18, p=0.036). These associations did not

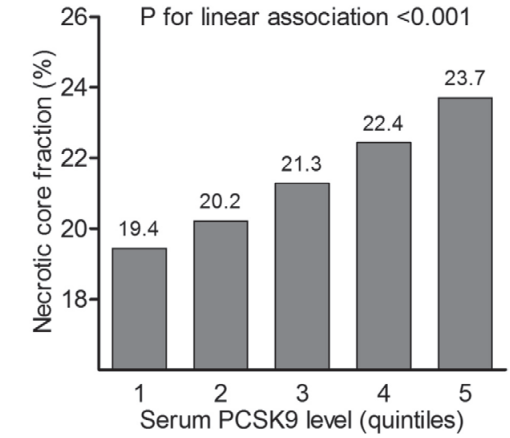

Figure 2. Association between serum PCSK9 level and fraction of coronary plaque that consists of necrotic core tissue
PCSK9, proprotein convertase substilisin/kexin type 9.

change materially after additional adjustment for serum LDL cholesterol levels (Table 2). The full univariate and multivariate predictors of necrotic core fraction are presented in Supplemental Table 1. Subgroup analysis showed that the positive association between serum PCSK9 levels and necrotic core fraction was present in all patient subgroups, including statin users and non-statin users as well as patients with low and high LDL cholesterol. (Figure 3). There was no significant heterogeneity in the β estimate between the evaluated patient subgroups.

PCSK9 level and cardiovascular outcome

Although this study was not primarily designed to investigate the association between serum PCSK9 levels and cardiovascular outcome, 1-year follow-up was available for (99.7%) of patients. A total of 28 patients died or had an ACS (definite culprit lesion-related events were not counted). Serum PCSK9 levels were significantly associated with the composite of death or ACS when PCSK9 was analyzed as a categorical variable (event rate 3.1% in patients with PCSK9 below median versus event rate 6.6% in patients with PCSK9 above median, p=0.049) (Supplemental Figure 2).

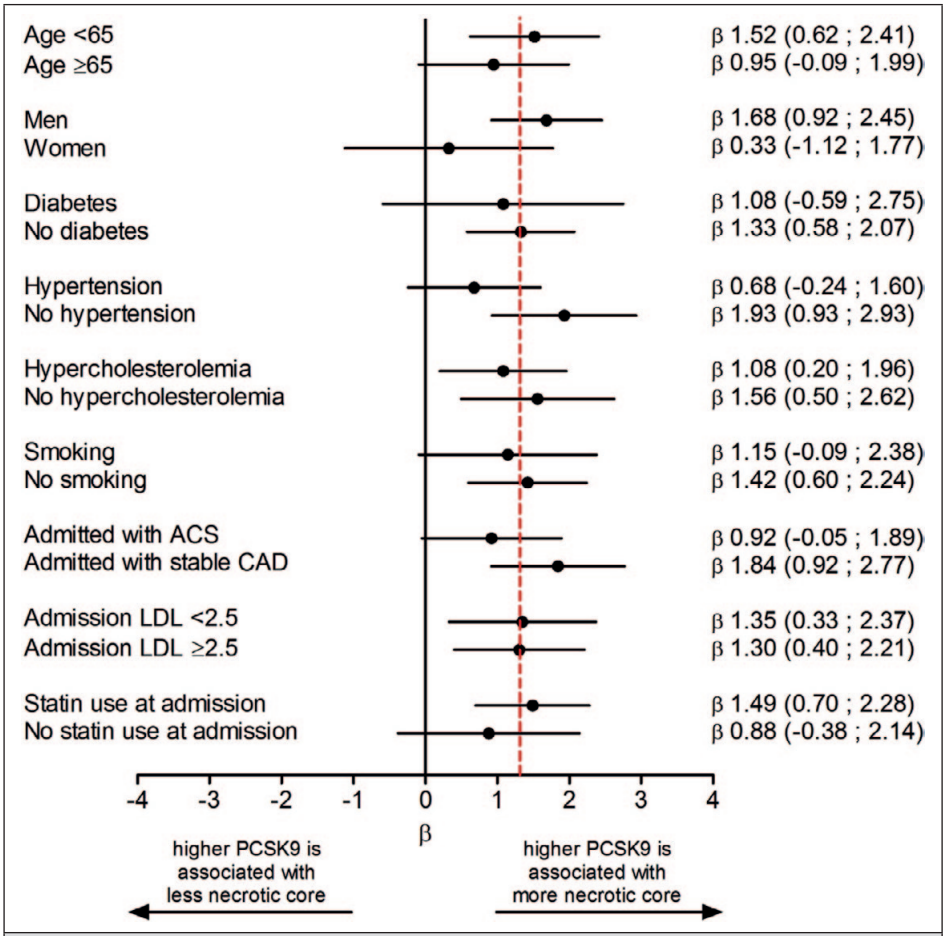

Figure 3. Association between PCSK9 level and necrotic core fraction stratified by patient subgroups

β (95% confidence interval) is increase in necrotic core fraction (%) per 100 μg/L increase in PCKS9. Red dotted line indicates the β estimate in the total study population.

ACS, acute coronary syndrome; CAD, coronary artery disease; LDL, low-density lipoprotein.

DISCUSSION

This study investigated the association of serum PCSK9 levels with the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS-VH imaging in patients with established CAD undergoing coronary angiography. The main finding was that higher serum PCSK9 levels were linearly associated with a higher necrotic core fraction in coronary atherosclerosis. This association was independent of serum LDL cholesterol levels and statin use, and was observed in all patient subgroups, including statin users and non-statin users as well as patients with low and high LDL cho-

lesterol. To the best of our knowledge, this is, as yet, the first study that has investigated the relation between serum PCSK9 levels and atherosclerotic plaque characteristics.

Serum PCSK9 levels vary between individuals.[21] The median PCSK9 level in our patient population with established CAD (270 µg/L) was higher than that in healthy individuals in previously published studies, for example in the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial (JUPITER) trial (71 µg/L).[21] Currently, PCSK9 is mostly known for its role in the regulation of cholesterol homeostasis.[2] It enhances the degradation of hepatic LDL receptors, resulting in an increase in LDL cholesterol levels.[2] Gain-of-function mutations in the PCSK9 gene are linked to familiar hypercholesterolemia, while loss-of-function mutations of the PCSK9 gene are linked to low LDL cholesterol levels and low cardiovascular risk, without currently known adverse effects on health.[22-25] Furthermore, statin treatment is known to increase PCSK9 levels by a negative feedback mechanism in response to lower cholesterol levels, making it even more interesting to investigate the effects of new PCSK9 inhibiting drugs on top of statin treatment.[21,26-28] Recent phase II clinical trials have reported promising results on serum LDL cholesterol levels by administration of monoclonal antibodies against PCSK9.[3-9]

Although a previous study did not find a significant association between serum PCSK9 levels and carotid intima-media thickness in healthy men,[29] other studies have suggested that PCSK9 may have a direct role in inflammatory processes contributing to atherosclerotic disease by mechanisms that are independent of LDL cholesterol levels.[2] Recent experimental studies have shown that PCSK9 is expressed in human atherosclerotic plaques.[30] PCSK9 enhances the expression of pro-inflammatory genes through activation of nuclear factor kappa beta (Nf-κB).[10] Inhibition of PCSK9 has been shown to suppress this pro-inflammatory pathway.[10] Furthermore, PCSK9 also targets apolipoprotein E receptor 2, which is a family member of the LDL receptor.[11] Degradation of apolipoprotein E receptor 2 is accompanied with loss of its known anti-inflammatory function.[11,31] Finally, PCSK9 is also associated with increased oxidized LDL-induced apoptosis of human endothelial cells, which may lead to endothelial dysfunction.[12] Inhibition of PCSK9 has been shown to suppress such endothelial apoptosis.[12] Our finding that serum PCSK9 levels were linearly associated with the amount of necrotic core by IVUS-VH imaging, independently of serum LDL cholesterol levels and in all patient subgroups (including statin users and non-statin users as well as patients with low and high LDL cholesterol), supports the hypothesis that PCSK9 has a direct role in plaque inflammation.

Although this study was not primarily designed to investigate the association between serum PCSK9 levels and cardiovascular outcome, a significant association between PCSK9 (below vs. above median level) and 1-year death or ACS was present. Previous studies have demonstrated that the presence of IVUS-VH-derived TCFA lesions and the amount of IVUS-VH-derived necrotic core tissue in coronary atherosclerosis are

both independent predictors of adverse coronary events.[14-16] Rupture of a TCFA lesion is believed to be a major cause of ACS.[32] Although we did not find an association between PCSK9 and the presence of IVUS-VH-derived TCFA lesions, we did find an association with its precursor, namely necrotic core. Plaque erosion due to chronic inflammation is another major cause of ACS.[33] It may be possible that PCSK9 has a role in plaque erosion through its involvement in the pro-inflammatory pathways and endothelial apoptosis as described above. The exact mechanism underlying the relation between PCSK9, the amount of necrotic core tissue and cardiovascular outcome (beyond its role in LDL cholesterol homeostasis) requires further elucidation in future research.

Some limitations of this study need to be acknowledged. Firstly, a single non-culprit coronary vessel was imaged in this study. This approach was eventually chosen to test the hypothesis that the phenotype of a non-culprit artery segment may indicate the patient's systemic atherosclerotic disease burden.[18] This hypothesis is supported by our previous finding that IVUS-VH imaging in only one non-culprit vessel appeared relevant for prognostication.[14] However, necrotic core-rich plaques (e.g. TCFA lesions) elsewhere in the coronary tree (including the culprit lesion) were not assessed in our study. This may have lead to an underestimation of the association between PCSK9 and necrotic core-rich plaques in the coronary tree. Secondly, repeated intracoronary imaging with IVUS-VH was not performed. Therefore, the association between PCSK9 and actual progression of necrotic core tissue and atherosclerotic plaque could not be investigated. Finally, this study was not primarily designed to investigate the association between PCSK9 and clinical outcome, and the number of clinical endpoints was relatively small.

CONCLUSIONS

In patients with established CAD, the range in serum PCSK9 levels is wide. Higher PCSK9 levels were linearly associated with a higher fraction and amount of IVUS-VH-derived necrotic core tissue in coronary atherosclerotic plaque. These associations were independent of serum LDL cholesterol levels and were observed in all patient subgroups, including statin users and non-statin users, as well as patients with low and high LDL cholesterol. Our results support the hypothesis that PCSK9 is directly involved in promoting inflammatory processes contributing to atherosclerosis by mechanisms independent of LDL cholesterol levels. Therefore, PCSK9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL cholesterol regulation (i.e. on top of statin treatment). Further research is warranted to investigate the effects of PCSK9 inhibiting therapies on the composition of atherosclerosis and on clinical outcome.

Funding

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Conflict of interest

None.

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SUPPLEMENTAL MATERIAL

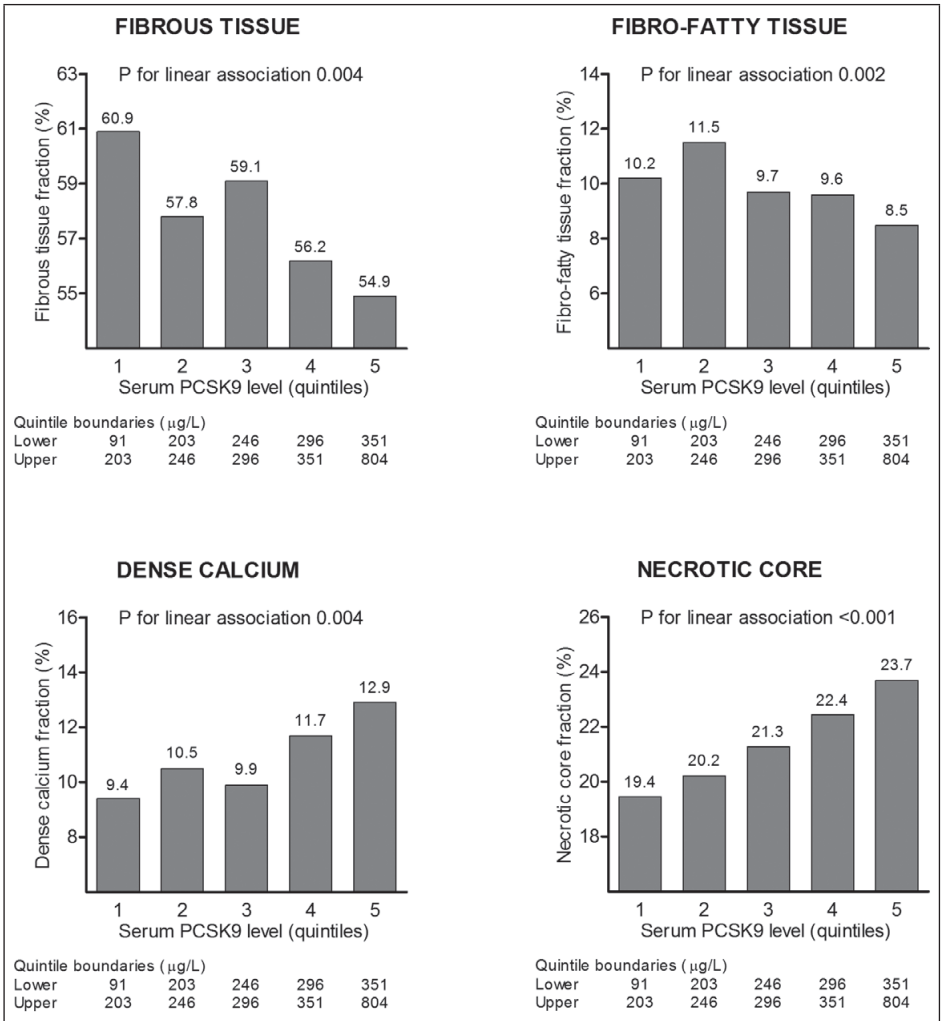
Supplemental Table 1. Predictors of necrotic core fraction

	Unadjusted	P-value	Adjusted*	P-value
Age	β -0.02 (-0.07;0.04)	0.62	β -0.02 (-0.08;0.05)	0.61
Gender (man)	β -0.73 (-2.3;0.79)	0.35	β -0.46 (-2.04;1.12)	0.57
Diabetes mellitus	β -0.86 (-2.60;0.89)	0.34	β -0.61 (-2.42;1.20)	0.51
Hypertension	β 0.60 (-0.72;1.91)	0.37	β 0.57 (-0.91;2.05)	0.45
Hypercholesterolemia	β 0.26 (-1.06;1.58)	0.70	β -0.28 (-1.87;1.31)	0.73
Smoking	β -0.25 (-1.70;1.20)	0.73	β -0.64 (-2.23;0.95)	0.43
ACS (vs stable CAD)	β -0.65 (-1.96;0.67)	0.34	β -0.95 (-2.40;0.50)	0.20
LDL cholesterol	β 0.28 (-0.33;0.90)	0.36	β 0.56 (-0.17-1.28)	0.13
Statin use	β 0.99 (-0.37;2.34)	0.15	β 1.71 (-0.02;3.44)	0.052
PCSK9†	β 1.31 (0.63;1.99)	<0.001	β 1.24 (0.55;1.94)	<0.001

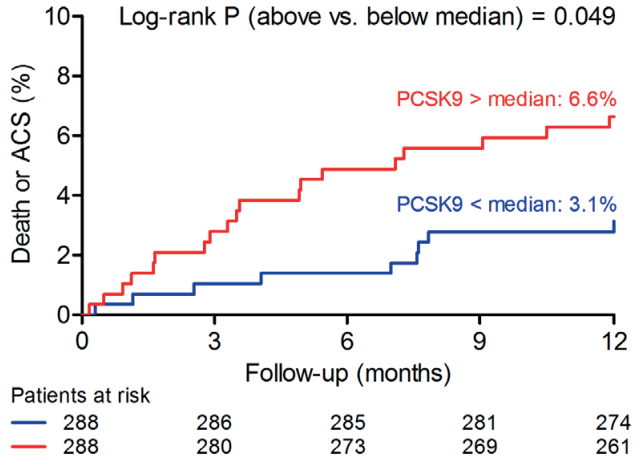
* Full model includes: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, clinical presentation (ACS or stable CAD), LDL cholesterol, statin use (registered at the time of hospital admission) and PCSK9.

† β (95% confidence interval) is increase in necrotic core fraction (%) per 100 μg/L increase in PCKS9.

ACS = acute coronary syndrome; CAD = coronary artery disease; LDL = low-density 9 = proprotein convertase substilisin/kexin type 9.



Supplemental Figure 1. Association between serum PCSK9 and plaque composition
PCSK9 = proprotein convertase substilisin/kexin type 9.



Supplemental Figure 2. Association of PCSK9 level with clinical outcome
Definite culprit lesion-related events were not counted as endpoint.
ACS = acute coronary syndrome.

CORONARY VULNERABILITY

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CIRCULATING CHEMOKINES IN RELATION TO CORONARY PLAQUE CHARACTERISTICS ON RADIOFREQUENCY INTRAVASCULAR ULTRASOUND AND CARDIOVASCULAR OUTCOME

Biomarkers 2014;19(7):611-9

ABSTRACT

Objective: To investigate relations of several circulating chemokines with extent and phenotype of coronary atherosclerosis and with 1-year clinical outcome.

Methods: Intravascular ultrasound virtual histology imaging of a coronary artery was performed in 581 patients. MCP-1, MIP-1α, MIP-1β and RANTES were measured in plasma.

Results: Higher MCP-1, MIP-1α and lower RANTES were associated with coronary plaque burden. Higher MCP-1, MIP-1α and lower RANTES were associated with the presence of IVUS-VH-derived thin-cap fibroatheroma lesions. RANTES was associated with major adverse cardiac events.

Conclusions: RANTES is a promising biomarker that is inversely associated with coronary plaque burden and vulnerability, as well as with death and ACS.

INTRODUCTION

Inflammation has been recognized as an important contributing factor in all phases of atherosclerosis.[1-3] In particular, inflammation is believed to play a crucial role in the development and rupture of vulnerable plaques, resulting in major cardiovascular problems such as myocardial infarction and stroke.[1-3] Circulating inflammatory biomarkers may potentially improve prognostication of patients with atherosclerotic cardiovascular disease.[4]

Chemokines are involved in the recruitment of various leukocytes, such as monocytes, macrophages and T lymphocytes, into the atherosclerotic plaque.[5,6] Monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β and regulated upon activation normal T cell expressed and secreted (RANTES) are typical C-C motif chemokines that have been studied extensively.[5,6] Several studies have shown that these chemokines have an important role throughout the entire atherosclerotic process from atherogenesis to plaque destabilization.[5,6] However, their clinical utility as biomarker remains unclear.[5,6] Furthermore, prospective data on associations of these biomarkers with in-vivo measurements of extensiveness, phenotype and vulnerability of coronary atherosclerosis is currently lacking. This study aims to evaluate the usefulness of MCP-1, MIP-1α, MIP-1β and RANTES by investigating their relations with intravascular ultrasound virtual histology (IVUS-VH)-derived measures of coronary plaque burden, quantity of necrotic core, and presence of thin-cap fibroatheroma lesions (TCFA), and by investigating their prognostic value for major adverse cardiac events.

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 in detail elsewhere.[7,8] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris (SAP) have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. 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 in ClinicalTrials.gov, number NCT01789411.

Data collection

Baseline characteristics of all patients were collected prospectively by trained research physicians. These physicians reviewed the medical charts of the patients at the time of inclusion in the study, and extracted variables regarding demographics, medical history, cardiovascular risk factors and procedural characteristics. Medical history and cardiovascular risk factors are a routine part of clinical patient assessment at the department of Cardiology. Thus, presence of diabetes mellitus, hypertension, hypercholesterolemia, history of renal insufficiency and history of heart failure were defined as a clinical diagnosis of these conditions as reported by the treating physician in the medical chart. Smoking was defined as current smoking, reported by the patient. Procedural characteristics were prospectively extracted from the catheterization report.

Biomarkers

Blood samples were drawn from the arterial sheath prior to the diagnostic coronary angiography or PCI procedure. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at temperature of −80°C within 2 hours after blood collection. MCP-1, MIP-1α, MIP-1β and RANTES were measured in the stored EDTA-plasma samples (n=570) using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA).

Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit coronary vessel. 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 IVUS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) that had no knowledge of clinical data. The IVUS gray-scale and IVUS radiofrequency analyses, also known as IVUS virtual histology, 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 (median interslice distance, 0.40 mm). 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 and is presented as a percentage. The composition of the atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.[9] A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive

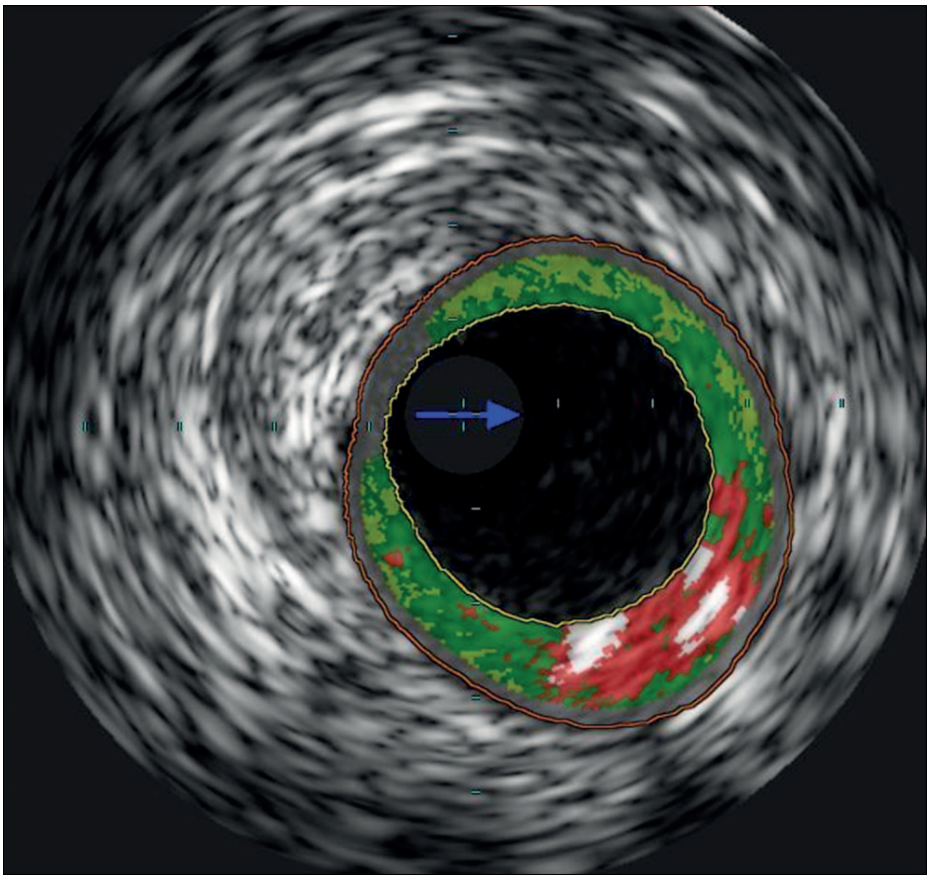

Figure 1. Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology
Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology is defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. White indicates dense calcium, light green indicates fibrofatty tissue, dark green indicates fibrous tissue.

frames. A thin-cap fibroatheroma (TCFA) lesion on IVUS-VH was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen (Figure 1).[10,11] TCFA lesions with a plaque burden of at least 70% were classified as large TCFA lesions.

Study endpoints

In this study, follow-up started at inclusion and lasted up to 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. 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.[12-14] 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 the 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 all-cause mortality, ACS or unplanned coronary revascularization. The secondary endpoint was defined as the composite of non-culprit lesion related or indeterminate all-cause mortality or ACS. Definite culprit lesion related events were excluded from the primary and secondary endpoints, because the pathophysiology of culprit lesions related events (e.g. in-stent restenosis or in-stent thrombosis) differs from our primary research focus on spontaneous plaque rupture leading to unanticipated, spontaneous MACE. The endpoints were adjudicated by a clinical event committee that had no knowledge of biomarkers and IVUS data.

Statistical analysis

The distributions of the continuous variables, including biomarker levels and the IVUS parameters, were tested for normality by visual examination of the histogram. Normally distributed continuous variables are presented as mean ± standard deviation (SD), while non-normally distributed continuous variables are presented as median and interquartile range (IQR). MCP-1, MIP-1α, MIP-1β and RANTES concentrations were not normally distributed and were therefore ln-tranformed for further analysis. Categorical variables are presented in percentages. We examined associations of biomarker concentrations with plaque burden and necrotic core fraction in the imaged coronary segment. Specifically, we calculated means of plaque burden and necrotic core fraction according to tertiles of biomarker concentration. To test for trends, we used linear regression analyses with continuous ln-transformed biomarker concentrations as the independent variable. The final results are presented as β (per SD increase in ln-transformed biomarker concentration) with 95% confidence interval (95% CI). Furthermore, we have examined the relation between biomarker concentrations and the presence of IVUS-VH derived TCFA lesions using logistic regression analyses with continuous ln-tranformed biomarker concentration as the independent variable. The final results are presented as odds ratio (OR) per SD increase in ln-transformed biomarker concentration with 95% CI.

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 log-rank test. Cox proportional hazards regression analyses were performed to evaluate the relationship between biomarker concentration and clinical endpoints. Biomarkers that were significantly associated with occurrence of MACE in univariable analysis were further evaluated in multivariable analyses. The variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, statin use, history of MI and indication for coronary angiography were considered as potential confounders and were entered into the full model. These covariates were a priori chosen, taking into account the number of events available. Subsequently, C-reactive protein (CRP) was also entered into the model to evaluate whether the associations between biomarkers and MACE were independent of CRP concentration. The final results are presented as hazard ratio (HR) per SD increase in ln-transformed biomarker concentration with 95% CI.

All statistical analyses were primarily performed in the overall study population. Heterogeneity in effect estimates between patients with ACS and patients with stable angina were examined using the Z-test for heterogeneity. If there was no heterogeneity, conclusions were based on the effect estimates belonging to the total study population. If there was significant heterogeneity between patients admitted with and without ACS, conclusions were based on effect estimates of the separate groups.

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 patients was 61.5 ± 11.4 years, 75.4% were men and 17.4% had diabetes mellitus (Table 1). Coronary angiography or PCI was performed for various indications: 159 (27.9%) patients had an acute myocardial infarction, 150 (26.3%) patients had unstable angina pectoris and 261 (45.8%) patients had stable angina pectoris. Some patients had biomarker concentrations beneath the lowest detection limit of the assay, which especially pertains to MIP-1α (measurable in 84% of patients). The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm. On basis of radiofrequency IVUS, a total of 239 (41.9%) patients had at least 1 IVUS-VH-derived TCFA, including 69 (12.1%) patients with at least 1 IVUS-VH-derived TCFA with a plaque burden ≥70%.

Table 1. Baseline characteristics

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Patient characteristics			
Age, years	61.5 ± 11.4	59.7 ± 11.9	63.6 ± 10.3
Men, n (%)	430 (75.4)	227 (73.5)	203 (77.8)
Diabetes mellitus, n (%)	99 (17.4)	40 (12.9)	59 (22.6)
Hypertension. n (%)	295 (51.8)	134 (43.4)	161 (61.7)
Hypercholesterolemia, n (%)	317 (55.6)	137 (44.3)	180 (69.0)
Smoking, n (%)	164 (28.8)	115 (37.2)	49 (18.8)
Positive family history, n (%)	293 (51.4)	140 (45.3)	153 (58.6)
Previous MI, n (%)	184 (32.3)	80 (25.9)	104 (39.8)
Previous PCI, n (%)	185 (32.5)	57 (18.4)	128 (49.0)
Previous CABG, n (%)	18 (3.2)	7 (2.3)	11 (4.2)
Previous stroke, n (%)	23 (4.0)	10 (3.2)	13 (5.0)
Peripheral artery disease, n (%)	36 (6.3)	12 (3.9)	24 (9.2)
History of renal insufficiency, n (%)	32 (5.6)	13 (4.2)	19 (7.3)
History of heart failure, n (%)	19 (3.3)	6 (1.9)	13 (5.0)
C-reactive protein, mg/L	2.1 [0.8-5.3]	2.8 [1.1-7.0]	1.5 [0.6-3.1]
Statin use, n (%)	359 (63.0)	146 (47.2)	213 (81.6)
Procedural characteristics			
Indication for catheterization			
Acute coronary syndrome, n (%)	309 (54.2)	309 (100)	0 (0)
Myocardial infarction, n (%)	159 (27.9)	159 (51.5)	0 (0)
Unstable angina pectoris, n (%)	150 (26.3)	150 (48.5	0 (0)
Stable angina pectoris, n (%)	261 (45.8)	0 (0)	261 (100)
Coronary artery disease			
No significant stenosis, n (%)	42 (7.4)	18 (5.8)	24 (9.2)
1-vessel disease, n (%)	301 (52.8)	168 (54.4)	133 (51.0)
2-vessel disease, n (%)	166 (29.1)	88 (28.5)	78 (29.9)
3-vessel disease, n (%)	61 (10.7)	35 (11.3)	26 (10.0)
PCI performed, n (%)	501 (87.9)	287 (92.9)	214 (82.0%)
Serum biomarker concentrations			
MCP-1, pg/ml *	91 [70-122]	92 [70-133]	88 [71-111]
MIP-1α, pg/ml †	16.0 [12.0-21.9]	15.0 [12.0-21.9]	17.0 [12.0-21.9]
MIP-1β, pg/ml *	123 [92-165]	130 [95-179]	114 [89-146]
RANTES, ng/ml ‡	11.0 [6.4-19.0]	14.0 [7.6-23.0]	9.1 [5.0-14.3]
IVUS segment characteristics			
Imaged coronary artery			
Left anterior descending, n (%)	204 (35.8)	117 (37.9)	87 (33.3)

Table 1 *(continued)*

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Left circumflex, n (%)	190 (33.3)	107 (34.6)	83 (31.8)
Right coronary artery, n (%)	176 (30.9)	85 (27.5)	91 (34.9)
Segment length, mm	44.1 [33.7-55.4]	43.9 [32.9- 54.1]	44.8 [34.2-57.2]
At least 1 TCFA	239 (41.9)	140 (45.3)	99 (37.9)
At least 1 TCFA with PB≥70%	69 (12.1)	32 (10.4)	37 (14.2)

* Measurable in >99% of patients; below limit of detection in <1% of patients.

† Measurable in 84% of patients; below limit of detection in 16% of patients.

‡ Measurable in all patients.

ACS indicates acute coronary syndrome; CABG, coronary artery bypass grafting; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; PB, plaque burden; PCI, percutaneous coronary intervention; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris; TCFA, thin-cap fibroatheroma.

Associations with coronary atherosclerosis

In patients who were admitted with stable angina pectoris, higher plasma MCP-1 concentrations were associated with higher coronary plaque burden (per SD increase of ln-transformed MCP-1: β=2.56, 95% CI 0.91-4.21, p=0.002) and a higher fraction of plaque consisting of necrotic core (per SD increase of ln-transformed MCP-1: β=1.14, 95% CI 0.02-2.25, p=0.045) (Table 2). Higher MCP-1 concentrations also seemed to be associated with the presence of IVUS-VH derived TCFA lesions (OR per SD increase in ln-transformed MCP-1 1.90, 95% CI 1.00-3.61, p=0.052) in patients who were admitted with stable angina pectoris (Table 3).

Higher MIP-1α concentrations were associated with higher plaque burden (per SD increase of ln-transformed MIP-1α: β=1.66, 95% CI 0.72-2.61, p=0.001), higher necrotic core fraction (per SD increase of ln-transformed MIP-1α: β=0.89, 95% CI 0.23-1.55, p=0.008) and with the presence of IVUS-VH derived TCFA lesions with plaque burden ≥70% (OR per SD increase in ln-transformed MIP-1α 1.75, 95% CI 1.09-2.81, p=0.021) in the total study population.

In patients who were admitted with ACS, lower RANTES concentrations were associated with higher plaque burden (per SD increase of ln-transformed RANTES: β=−1.57, 95% CI −2.94;−0.20, p=0.025) (Figure 2). Furthermore, lower RANTES concentrations also seemed to be associated with the presence of IVUS-VH derived TCFA lesions with plaque burden ≥70% in the overall patient population (OR per SD increase in ln-transformed RANTES 0.76, 95% CI 0.57-1.02, p=0.067).

Table 2. Associations with plaque burden and necrotic core fraction in imaged coronary segment

	Total study population (n=570)				ACS patients (n=309)				SAP patients (n=261)				Hetero-geneity
	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*P*
Mean values of plaque burden (%)													
MCP-1	38.0 ± 11.0	37.8 ± 11.3	38.9 ± 12.4	0.46	38.4 ± 11.9	35.7 ± 11.0	36.9 ± 12.5	0.49	37.7 ± 9.9	40.2 ± 10.7	41.0 ± 12.3	0.002	0.004
MIP-1α	36.9 ± 10.8	37.8 ± 9.8	39.0 ± 11.9	0.001	35.1 ± 10.6	36.5 ± 10.1	39.3 ± 12.2	0.001	38.8 ± 10.9	39.8 ± 9.0	38.6 ± 11.7	0.38	0.10
MIP-1β	36.7 ± 11.2	39.0 ± 11.5	39.0 ± 11.8	0.31	36.5 ± 12.2	38.6 ± 11.4	36.0 ± 11.8	0.84	37.3 ± 10.1	39.5 ± 11.9	42.1 ± 10.8	0.015	0.071
RANTES	39.5 ± 10.9	37.7 ± 12.2	37.5 ± 11.4	0.089	38.8 ± 11.4	37.3 ± 12.0	34.9 ± 11.8	0.025	39.4 ± 10.3	38.3 ± 12.0	41.2 ± 10.8	0.32	0.022
Mean values of necrotic core fraction (%													
MCP-1	21.3 ± 8.1	21.3 ± 7.3	21.6 ± 8.8	0.84	22.6 ± 8.4	21.1 ± 8.2	21.5 ± 9.2	0.32	19.6 ± 7.3	21.6 ± 6.5	21.9 ± 8.1	0.045	0.027
MIP-1α	21.1 ± 7.6	21.6 ± 7.2	21.5 ± 8.7	0.008	21.7 ± 7.9	21.0 ± 7.4	23.0 ± 9.3	0.009	20.1 ± 7.2	22.4 ± 6.7	19.9 ± 7.7	0.33	0.27
MIP-1β	21.4 ± 8.0	21.4 ± 7.5	21.4 ± 8.7	0.76	21.9 ± 8.1	21.3 ± 8.0	22.0 ± 9.6	0.84	20.8 ± 7.8	21.5 ± 6.1	20.9 ± 8.1	0.91	0.83
RANTES	21.8 ± 7.3	21.1 ± 9.1	21.4 ± 7.8	0.53	22.8 ± 8.1	21.6 ± 9.1	20.8 ± 8.5	0.17	21.0 ± 6.4	20.4 ± 8.3	21.8 ± 7.4	0.81	0.24

P-values were obtained with linear regression analyses with continuous ln-transformed biomarker concentration as independent variable.

* Tertiles of biomarker levels.

ACS indicates acute coronary syndrome; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

Table 3. Associations with presence of intravascular ultrasound virtual histology-derived thin-cap fibroatheroma lesions

	Total study population (n=570)		ACS patients (n=309)		SAP patients (n=261)		Hetero-geneity
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	P
Presence of at least 1 thin-cap fibroatheroma							
MCP-1	1.03 (0.74-1.45)	0.85	0.77 (0.51-1.17)	0.22	1.90 (1.00-3.61)	0.052	0.022
MIP-1α	0.87 (0.63-1.21)	0.42	0.94 (0.61-1.42)	0.75	0.83 (0.49-1.39)	0.47	0.72
MIP-1β	1.16 (0.85-1.60)	0.36	1.18 (0.79-1.76)	0.42	0.97 (0.55-1.70)	0.91	0.69
RANTES	0.97 (0.80-1.18)	0.75	0.87 (0.66-1.15)	0.33	0.98 (0.72-1.33)	0.90	0.57
Presence of at least 1 thin-cap fibroatheroma with plaque burden ≥70%							
MCP-1	1.23 (0.75-2.04)	0.41	0.94 (0.48-1.83)	0.86	2.16 (0.95-4.93)	0.067	0.12
MIP-1α	1.75 (1.09-2.81)	0.021	2.15 (1.13-4.09)	0.020	1.29 (0.63-2.66)	0.49	0.30
MIP-1β	0.89 (0.54-1.47)	0.66	0.91 (0.47-1.78)	0.79	1.01 (0.46-2.20)	0.98	0.85
RANTES	0.76 (0.57-1.02)	0.067	0.73 (0.47-1.15)	0.17	0.84 (0.55-1.28)	0.41	0.67

Odds ratios are per standard deviation increase in ln-transformed biomarker concentration.
ACS indicates acute coronary syndrome; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

Major adverse cardiac events

Vital status was acquired for 569 (99.8%) patients. Response rate of the questionnaires that were sent to all living patients was 92.3%. After 1 year of follow-up, 56 patients had at least 1 event (Supplemental table 1). 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 either culprit lesion related or non-culprit lesion related and were therefore classified as having an indeterminate event. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year composite of non-culprit lesion related or indeterminate death, ACS or unplanned coronary revascularization were 0.7%, 4.7%, and 7.9%, respectively. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year composite of non-culprit lesion related or indeterminate death or ACS were 0.7%, 3.2%, and 4.9%, respectively.

Associations with non-culprit lesion related and indeterminate events

In univariable analysis, RANTES (HR per SD increase of ln-transformed RANTES 0.67, 95% CI 0.50-0.89, p=0.005) was associated with occurrence of the primary endpoint of non-culprit lesion related and indeterminate MACE during follow-up (Table 4, Figure 2). There was no heterogeneity in the hazard ratio estimate between ACS patients and patients with stable angina (heterogeneity p=0.39). RANTES (HR per SD increase of ln-transformed RANTES 0.64, 95% CI 0.45-0.91, p=0.013) was also significantly associated

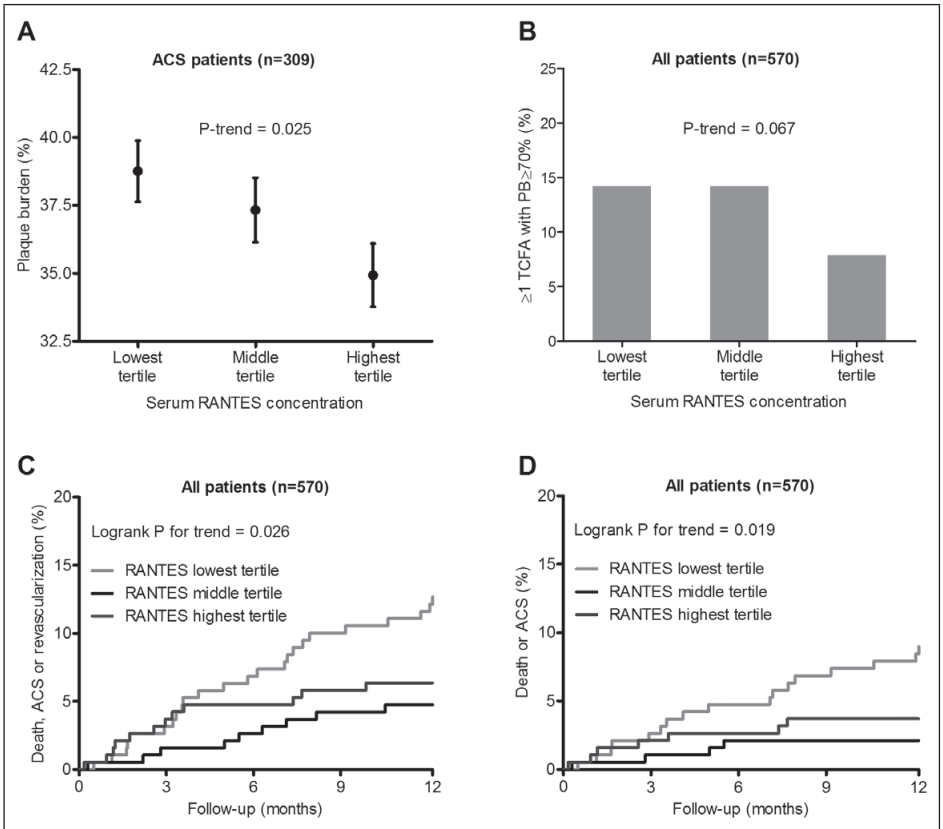

Figure 2. Associations of circulating RANTES concentrations with coronary atherosclerosis and clinical outcome

A. Association with intravascular ultrasound-derived measures of coronary plaque burden in patients admitted with acute coronary syndrome.

B. Association with presence of thin-cap fibroatheroma lesions with plaque burden ≥70% as assessed by intravascular ultrasound virtual histolgy.

C. Association with occurrence of non-culprit lesion related and indeterminate death, acute coronary syndrome or coronary revascularization. The lowest RANTES tertile was associated with the highest event rate (lowest tertile vs. middle tertile p=0.006; lowest tertile vs. highest tertile p=0.042; middle tertile vs. highest tertile p=0.50; logrank p for trend=0.026).

D. Association with occurrence of non-culprit lesion related and indeterminate death or acute coronary syndrome. The lowest RANTES tertile was associated with the highest event rate (lowest tertile vs. middle tertile p=0.004; lowest tertile vs. highest tertile p=0.039; middle tertile vs. highest tertile p=0.86; logrank p for trend=0.019).

ACS indicates acute coronary syndrome; PB, plaque burden; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; TCFA, thin-cap fibroatheroma.

with the composite of non-culprit lesion related and indeterminate death or ACS only. After adjustment for conventional cardiovascular risk factors in multivariable analysis, RANTES remained independently predictive for non-culprit lesion related and inde-

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

	Total study population (n=570)		ACS patients (n=309)		SAP patients (n=261)		Hetero-geneity
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*P*
Major adverse cardiac events (primary endpoint)							
MCP-1	0.87 (0.64-1.18)	0.37	0.81 (0.55-1.20)	0.29	1.00 (0.61-1.65)	1.00	0.51
MIP-1α	1.13 (0.85-1.49)	0.40	1.16 (0.82-1.66)	0.40	1.06 (0.69-1.64)	0.80	0.74
MIP-1β	1.00 (0.74-1.34)	0.99	1.15 (0.82-1.62)	0.42	0.82 (0.50-1.34)	0.42	0.26
RANTES	0.67 (0.50-0.89)	0.005	0.77 (0.50-1.18)	0.23	0.59 (0.40-0.88)	0.009	0.39
Composite of death or acute coronary syndrome (secondary endpoint)							
MCP-1	0.73 (0.48-1.09)	0.12	0.74 (0.47-1.16)	0.19	0.69 (0.31-1.53)	0.36	0.88
MIP-1α	1.11 (0.77-1.58)	0.58	1.12 (0.73-1.70)	0.61	1.11 (0.59-2.09)	0.74	0.99
MIP-1β	1.11 (0.78-1.57)	0.57	1.34 (0.98-1.84)	0.071	0.48 (0.24-0.98)	0.043	0.010
RANTES	0.64 (0.45-0.91)	0.013	0.58 (0.36-0.94)	0.028	0.62 (0.35-1.10)	0.10	0.86

Hazard ratios are per standard deviation increase in ln-transformed biomarker concentration.
MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

terminate MACE (HR per SD increase of ln-transformed RANTES 0.69, 95% CI 0.52-0.93, p=0.016) and for non-culprit lesion related and indeterminate death or ACS only (HR per SD increase of ln-transformed RANTES 0.60, 95% CI 0.41-0.88, p=0.010) (Table 5). RANTES also remained independently associated with MACE (HR per SD increase of ln-transformed RANTES 0.69, 95% CI 0.51-0.93, p=0.014) and the composite of death or ACS

Table 5. Multivariable analysis on non-culprit lesion related and indeterminate major adverse cardiac events

	Adjusted for age and gender		Adjusted for age, gender and indication for angiography		Adjusted for conventional risk factors and indication for angiography*		Adjusted for conventional risk factors, indication for angiography and CRP*	
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Major adverse cardiac events (primary endpoint)								
RANTES	0.72 (0.54-0.96)	0.024	0.71 (0.53-0.95)	0.023	0.69 (0.52-0.93)	0.016	0.69 (0.51-0.93)	0.014
Composite of death or acute coronary syndrome (secondary endpoint)								
RANTES	0.69 (0.48-0.99)	0.046	0.64 (0.44-0.93)	0.021	0.60 (0.41-0.88)	0.010	0.59 (0.40-0.88)	0.010

Hazard ratios are per standard deviation increase in ln-transformed biomarker concentration.
* Conventional risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, statin use and history of myocardial infarction.
CRP indicates C-reactive protein; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted.

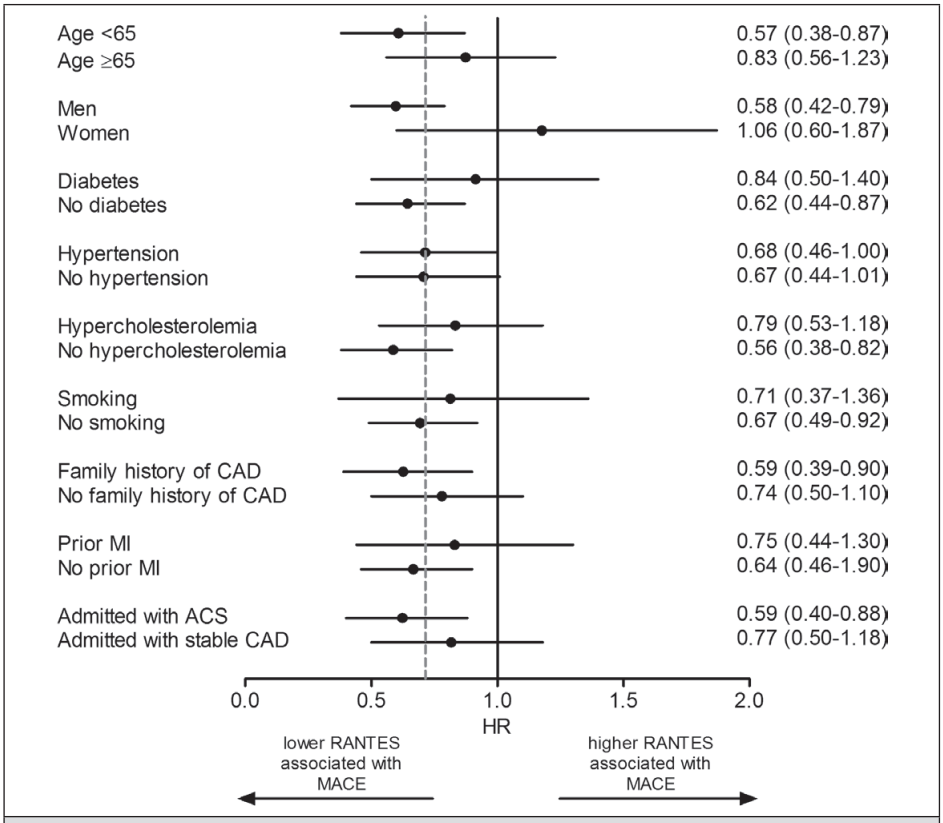

Figure 3. Association between RANTES level and major adverse cardiac events stratified by patient subgroups

Hazard ratios (95% confidence intervals) are per standard deviation increase in ln-transformed RANTES concentration. Dotted line indicates the hazard ratio estimate in the total study population.

ACS indicates acute coronary syndrome; CAD, coronary artery disease; HR, hazard ratio; MI, myocardial infarction; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted.

only (HR per SD increase of ln-transformed RANTES 0.59, 95% CI 0.40-0.88, p=0.010) after additional adjustment for baseline CRP levels. Subgroup analysis showed that the inverse association between RANTES level and MACE was present in all patient subgroups (Figure 3). There was no significant heterogeneity in the hazard ratio estimate between the evaluated patient subgroups.

DISCUSSION

This study investigated the relations of circulating chemokine concentrations with extensiveness of coronary atherosclerosis, amount of necrotic core, the presence of

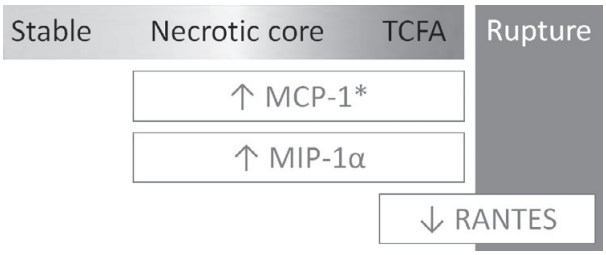

Figure 4. Circulating chemokine concentrations as biomarkers for phenotype of coronary atherosclerosis and risk of plaque rupture

Hypothesized model based on findings in this study. Plasma MCP-1, MIP-1α and RANTES concentrations were all associated with coronary plaque burden and different plaque phenotypes. RANTES was associated with major adverse cardiac events that were most probably caused by plaque rupture.

* Only for patients with stable angina pectoris.

↑ indicates higher biomarker concentrations.

↓ indicates lower biomarker concentrations.

MCP-1 indicates monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; TCFA, thin-cap fibroatheroma.

IVUS-VH derived TCFA lesions and occurrence of future major adverse cardiac events in patients who underwent coronary angiography for ACS or stable angina pectoris. To our best knowledge, this is the first study that correlates circulating chemokines with in-vivo measurements of coronary atherosclerosis using IVUS-VH. Higher plasma MCP-1, MIP-1α, and lower RANTES concentrations were all associated with higher coronary plaque burden and more advanced plaque phenotypes as determined by IVUS-VH (Figure 4). However, only RANTES was found to be independently predictive for the occurrence of MACE, particularly of death and ACS.

Chemokines are small cytokines that have the ability to induce directed chemotaxis of nearby leukocytes. MCP-1, MIP-1α, MIP-1β and RANTES belong to the C-C motif chemokine ligand (CCL) family and are also known as CCL2, CCL3, CCL4 and CCL5, respectively.[5,6] Pathologic studies have shown that these chemokines are highly expressed in atherosclerotic plaques.[15-17] Animal studies have shown that these chemokines are actively involved in atherogenesis and plaque destabilization.[5,6] Furthermore, several epidemiological studies have indicated that serum or plasma levels of MCP-1, MIP-1α, MIP-1β and RANTES may predict future cardiac events.[5] However, their clinical utility as biomarker for cardiovascular risk stratification remains unclear.[5,6] We sought to further elucidate the correlations of circulating chemokine concentrations with in-vivo measurements of extensiveness, phenotype and vulnerability of coronary atherosclerosis by using IVUS-VH.

Grey-scale IVUS allows for in-vivo measurements of coronary plaque burden. Additionally, radiofrequency IVUS allows for differentiation of the composition of the athero-

sclerotic plaque and is therefore also known as IVUS-VH.[10] Necrotic core is often found in the more advanced and rupture-prone plaques.[18] The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study has demonstrated that TCFA lesions as determined by IVUS-VH are associated with MACE.[19] The strong and independent associations (adjusted hazard ratios ranging from 1.79 to 3.35) of IVUS-VH-derived TCFA with MACE emphasize its biological importance.[18-20] However, there are several reasons why IVUS is currently not suitable for use as diagnostic and prognostic tool in the overall population of patients with coronary artery disease.[19] Its invasiveness is probably the most important limitation in this respect. Therefore, circulating biomarkers may have an important role in cardiovascular risk assessment.

In our study, lower plasma RANTES concentrations were independently associated with adverse outcomes during 1 year of follow-up. The association was independent of CRP. Its association with acute cardiac events (death or ACS; HR 0.59) seemed to be even stronger than with all major adverse cardiac events (death, ACS or unplanned coronary revascularization; HR 0.69). This may indicate that RANTES is especially predictive for plaque rupture rather than plaque growth. Our finding that low serum RANTES concentrations, rather than high, are associated with adverse coronary events may seem counterintuitive, since animal studies have shown that RANTES and its receptor are actively involved in atherogenesis and that RANTES was found to be highly expressed within atheromous lesions.[6,21] However, the inverse associations of RANTES may be explained by increased deposition of RANTES on the vascular endothelium, resulting in lower free circulating serum concentrations.[22,23] The inverse associations of RANTES are also consistent with observations from previous studies. A large case-control study reported that serum RANTES levels were lower in coronary heart disease patients compared with age- and gender-matched controls.[22] Another study reported that low plasma RANTES levels were independently associated with cardiac mortality in 389 male patients who underwent coronary angiography.[23] Such an association was not found in a population-based case-cohort study that included 363 individuals with incident coronary events and 1908 non-cases.[24]

We found that higher plasma MCP-1 concentrations were associated with higher coronary plaque burden in patients who were admitted with stable angina pectoris. These findings are in line with a previous study that measured MCP-1 concentrations in blood from the coronary sinus and found that these levels were associated with the extent of coronary atherosclerosis as assessed on the coronary angiogram.[25] Although we observed that high MCP-1 concentrations were associated with a more advanced plaque phenotype (i.e. higher necrotic core fraction) and with the presence of IVUS-VH derived TCFA lesions, MCP-1 was not predictive for future events. Previous epidemiological studies have shown that the ability of MCP-1 to predict subclinical coronary artery disease is somewhat disappointing, but that MCP-1 may have some value in predicting

cardiovascular events in patients with overt coronary artery disease.[5] For example, a previous study found that MCP-1 was independently associated with the composite of death or myocardial infarction in a large cohort of 4244 patients with ACS.[26] This study also demonstrated that high MCP-1 values at 4 months after the initial ACS were still predictive for long-term mortality afterwards. A major difference with our study is that both culprit lesion related and non-culprit lesion related events were included in their study endpoints, while definite culprit lesion related events were excluded from our study endpoints. Furthermore, we may have lacked statistical power to detect the previously reported association.

MIP-1α has been studied less extensively. We found that MIP-1α was associated with coronary plaque burden, necrotic core fraction and with the presence of large TCFA lesions on IVUS-VH. However, we did not observe a correlation between MIP-1α concentration and occurrence of MACE. Another study, however, found that MIP-1α was predictive for recurrent ACS in a relatively small cohort of 54 patients with unstable angina pectoris.[27] Further research is required to elucidate the role of MIP-1α in patients with coronary artery disease.

CONCLUSIONS

Higher circulating MCP-1, MIP-1α, and lower RANTES concentrations were associated with a higher extent, a more advanced phenotype and a higher vulnerability of coronary atherosclerosis. Such associations were not present for MIP-1β. In addition, RANTES was independently associated with occurrence of MACE, particularly of death and ACS. Its prognostic value was similar in patients with and without ACS. Its inverse associations are consistent with observations from previous studies and may be explained by increased deposition of RANTES on the endothelium, resulting in lower free circulating concentrations. The findings in this study demonstrate that RANTES may be a useful biomarker for assessment of cardiovascular risk. Further research on the incremental prognostic value of RANTES over established clinical covariates in large, prospective studies is warranted.

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SUPPLEMENTAL TABLES

Supplemental table 1. Patients with major adverse cardiac events

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

CORONARY VULNERABILITY

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CIRCULATING CYTOKINES IN RELATION TO THE EXTENT AND COMPOSITION OF CORONARY ATHEROSCLEROSIS

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ABSTRACT

Objective: We investigated whether concentrations of TNF-α, TNF-β, TNF-receptor 2, interferon-γ, IL-6, IL-8, IL-10 and IL-18 are associated with cardiovascular outcome, as well as extent and composition of coronary atherosclerosis determined by grayscale and virtual histology (VH)-intravascular ultrasound (IVUS).

Methods: Between 2008-2011, IVUS(-VH) imaging of a non-culprit coronary artery was performed in 581 patients (stable angina pectoris (SAP), n=261; acute coronary syndrome (ACS), n=309) undergoing coronary angiography from the ATHEROREMO-IVUS study. Coronary plaque burden and VH-derived thin-cap fibroatheroma (TCFA) lesions were assessed. Major adverse cardiac events (MACE: all-cause mortality, ACS, unplanned coronary revascularization) were registered during 1-year follow-up. We applied linear and logistic regression.

Results: TNF-α levels were positively associated with plaque burden (beta (β) [95%CI]: 4.45 [0.99-7.91], for highest vs lowest TNF-α tertile) and presence of VH-TCFA lesions (odds ratio (OR) [95%CI] 2.30 (1.17-4.52), highest vs lowest TNF-α tertile) in SAP patients. Overall, an inverse association was found between IL-10 concentration and plaque burden (β [95%CI]: −1.52 [−2.49 – −0.55], per Ln(pg/mL) IL-10) as well as IL-10 and VH-TCFA lesions with plaque burden ≥70% (OR: 0.31 [0.12-0.80],highest vs lowest IL-10 tertile). These effects did not reach statistical significance in the separate SAP and ACS groups. Fifty-six (9.8%) patients had MACE. No statistically significant associations were present between biomarkers and MACE.

Conclusion: Higher circulating TNF-α was associated with higher plaque burden and VH-TCFA lesions in SAP patients. Lower circulating IL-10 was associated with higher plaque burden and large VH-TCFA lesions. These in-vivo findings suggest a role for these cytokines in extent and vulnerability of atherosclerosis.

INTRODUCTION

Inflammation is known to play a major role in atherosclerosis[1-3].The development of atherosclerosis includes, among others, expression of adhesion molecules by inflamed endothelium, migration of leukocytes into the intima, uptake of modified lipoprotein particles, and formation of lipid-laden macrophages[4]. During the evolution of atherosclerotic lesions, T-lymphocytes join the macrophages in the intima[4]. This T-cell infiltrate produces proinflammatory cytokines (including tumor necrosis factors (TNFs), interferons (IFNs), and interleukins (ILs)), but may also stimulate a T helper cell type 2 (Th2) response which can promote anti-inflammatory actions (and cytokines such as IL-10 and transforming growth factor β) [2, 5]. This dual role of cytokines is believed to control the subsequent development and destabilization of arherosclerotic plaques in coronary (among other) arteries[6], potentially leading to plaque rupture or erosion and ultimately resulting in adverse clinical events such as myocardial infarction or sudden cardiac death [7].

While previous research has provided ample insights into the signalling cascades of cytokines and their roles in the pathogenesis of atherosclerosis, studies on the associations of cytokines with in-vivo determined extent and particularly composition of coronary atherosclerosis are currently scarce. Cytokines are located both inside the affected vessel walls and in the circulation [8]. We hypothesize that circulating cytokines are associated with in-vivo measures of plaque burden and features of plaque vulnerability, and consequently may be useful for clinical risk stratification with regard to cardiovascular outcome.

The aim of this study is to examine the associations of the cytokines TNF-α, TNF-β, interferon γ (IFNγ), IL-6, IL-8, IL-10 and IL-18 and of circulating TNF receptor 2 (TNF R2) with the extent and composition of coronary atherosclerosis as determined in-vivo by intravascular ultrasound (IVUS) and IVUS-virtual histology (IVUS-VH), in a non-culprit vessel in patients undergoing coronary angiography. Furthermore, the prognostic value of the cytokines for major adverse cardiac events (MACE) in these patients is studied.

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[9]. In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS; n=309) or stable angina pectoris (SAP; n=261) have been included from November

2008 to January 2011 in the Erasmus MC, Rotterdam, the Netherlands. Intravascular ultrasound (IVUS) of a non-culprit coronary artery was performed subsequent to angiography. The ATHEROREMO-IVUS study has been approved by the human research ethics committee of Erasmus MC, Rotterdam, the Netherlands. Written informed consent was obtained from all included patients and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Biomarkers

Blood samples were drawn from the arterial sheath prior to the diagnostic coronary angiography or PCI procedure, and were available in 570 patients for the current study. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at a temperature of −80°C within two hours after blood collection.

C-reactive protein (CRP) was measured in serum samples using a immunoturbidimetric high sensitivity assay (Roche Diagnostics Ltd., Rotkreuz, Switzerland) on the Cobas 8000 modular analyzer platform (Roche Diagnostics Ltd., Rotkreuz, Switzerland). These analyses were performed in the clinical laboratory of Erasmus MC.

Frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of −80°C) to Myriad RBM, Austin, Texas, USA, where the concentrations of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 were determined using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA). While TNF-α, TNF R2, IL-6, and IL-8 were determined in the full cohort of 570 patients, TNF-β, INFγ, IL-10 and IL-18, were determined in a random subset of 473 patients. This difference in numbers resulted from batch-wise handling of the samples in combination with an update of the composition of the multiplex assay by the manufacturer in-between two batches. None of the biomarker laboratories had knowledge of clinical or intracoronary imaging data.

Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit, non-treated, coronary vessel, without significant luminal narrowing. 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 IVUS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) that had no knowledge of clinical or biomarker data. The IVUS gray-scale and IVUS radiofrequency analyses, also known as IVUS virtual histology (IVUS-VH), 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 (median

interslice distance, 0.40 mm). Extent and phenotype of the atherosclerotic plaque were assessed.

Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area and is presented as a percentage. A coronary lesion was defined as a segment with a plaque burden of 40% in at least three consecutive frames[9]. Using IVUS-VH, the composition of the atherosclerotic plaque was characterized into 4 different types: fibrous, fibro-fatty, dense calcium and necrotic core [10]. A VH-IVUS-derived thin-cap fibroatheroma (TCFA) lesion was defined as a lesion with presence of > 10% confluent necrotic core in direct contact with the lumen[11].

Clinical study endpoints

In this study, follow-up lasted up to 1 year post angiography. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed. 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.[12-14] Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). The primary endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. The endpoints were adjudicated by a clinical event committee that had no knowledge of biomarkers and IVUS data.

Statistical analysis

Categorical variables are presented in percentages. The distributions of continuous variables, including biomarker levels and IVUS parameters, were examined for normality by visual inspection of the histogram and calculation of the skewness coefficient. Normally distributed continuous variables are presented as mean ± standard deviation (SD), while non-normally distributed continuous variables are presented as median and interquartile range (IQR). For reasons of uniformity, all biomarkers are presented as median (IQR).

In further analyses, biomarker concentrations were examined both as continuous and as categorical variables (the latter by dividing the variables into tertiles). Biomarkers with a non-normal distribution were ln-transformed. Biomarkers in which the concentrations were too low to detect in more than 20% of the patients, were not examined as continuous variables. They were examined as tertiles, or else as dichotomous variables (measurable vs not measurable).

To take into account possible effect modification by indication for coronary angiography, we performed all analyses separately in patients with SAP and patients with

ACS. We also present the results for the full cohort, in order to evaluate the effect of higher statistical power in those cases where associations were present in both groups of patients.

First, we examined associations of biomarker concentrations with the extent of atherosclerosis according to IVUS. We applied linear regression analyses with biomarker concentrations as the independent variable (ln-transformed or categorized when appropriate) and segmental plaque burden in the imaged coronary segment as the dependent variable. The results are presented as βs (per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration) with 95% confidence intervals (95% CI). Subsequently, we examined the associations between biomarker concentrations and composition of atherosclerosis, specifically the presence of VH-TCFA lesions as well as VH-TCFA lesions with plaque burden ≥ 70%. We used logistic regression analyses with biomarker concentrations as the independent variable (ln-transformed or categorized when appropriate). The results are presented as odds ratios (ORs) per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

Moreover, we examined associations of biomarker concentrations with MACE during 1 year follow-up. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. We used Cox proportional hazard regression analyses with biomarker concentration as the independent variable (ln-transformed or categorized when appropriate). The results are presented as hazard ratios (HRs) per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

First, all above-described analyses were performed univariably. Subsequently, we adjusted for age, gender, indication for coronary angiography, diabetes, hypertension and CRP.

All data were analyzed with SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics are summarized in Table 1. Mean age was 61.5 ± 11.4 years and 75.4% were men. Coronary angiography or PCI was performed for several indications: 159 (27.9%) patients had an acute myocardial infarction, 150 (26.3%) patients had unstable angina pectoris and 261 (45.8%) had SAP. The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm. Based on IVUS-VH, a total of 239 (41.9%)

Table 1. Baseline characteristics.

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Patient characteristics			
Age, years (mean±SD)	61.5 ± 11.4	59.7 ± 11.9	63.6 ± 10.3
Men, n(%)	430 (75.4)	227 (73.5)	203 (77.8)
Diabetes Mellitus, n(%)	99 (17.4)	40 (12.9)	59 (22.6)
Hypertension, n (%)	295 (51.8)	134 (43.4)	161 (61.7)
Hypercholesterolemia, n(%)	317 (55.6)	137 (44.3)	180 (69.0)
Smoking, n (%)	164 (28.8)	115 (37.2)	49 (18.8)
Positive family history, n (%)	293 (51.5)	140 (45.5)	153 (58.6)
Previous MI, n (%)	184 (32.3)	80 (25.9)	104 (39.8)
Previous PCI, n (%)	185 (32.5)	57 (18.4)	128 (49.0)
Previous CABG, n (%)	18 (3.2)	7 (2.3)	11 (4.2)
Previous stroke, n (%)	23 (4.0)	10 (3.2)	13 (5.0)
Peripheral artery disease, n (%)	36 (6.3)	12 (3.9)	24 (9.2)
History of renal insufficiency, n (%)	32 (5.6)	13 (4.2)	19 (7.3)
History of heart failure, n (%)	19 (3.3)	6 (1.9)	13 (5.0)
Procedural characteristics			
Indication for coronary angiography			
Acute coronary syndrome, n (%)	309 (54.2)	309 (100)	0 (0)
Myocardial infarction, n (%)	159 (27.9)	159 (51.5)	0 (0)
Unstable angina pectoris, n(%)	150 (26.3)	150 (48.5)	0 (0)
Stable angina pectoris, n (%)	261 (45.8)	0 (0)	261 (100)
Coronary artery disease			
No significant stenosis, n (%)	42 (7.4)	18 (5.8)	24 (9.2)
1-vessel disease, n (%)	301 (52.8)	168 (54.4)	133 (51.0)
2-vessel disease, n (%)	166 (29.1)	88 (28.5)	78 (29.9)
3-vessel disease, n (%)	61 (10.7)	35 (11.3)	26 (10.0)
PCI performed, n (%)	501 (87.9)	287 (92.9)	214 (82.0)
IVUS characteristics			
Segment length (mm), median (IQR)	44.1 (33.7-55.4)	43.9 (32.9-54.1)	44.8 (34.2-57.2)
Plaque burden (%), median (IQR)	39.2 (30.0-46.4)	37.2 (28.0-45.5)	40.2 (31.8-47.8)
Presence of VH-TCFA, n(%)	239 (41.9)	140 (45.3)	99 (37.9)
Presence of VH-TCFA with PB ≥ 70%, n(%)	69 (12.1)	32 (10.4)	37 (14.2)
Serum biomarker concentrations			
C-reactive protein (mg/L), median (IQR)	2.1 [0.8-5.3]	2.8 [1.1-7.0]	1.5 [0.6-3.1]
Tumor Necrosis Factor α (pg/mL) median (IQR)[+]	2.0 [1.4-2.9]	1.8 [1.4-2.6]	2.0 [1.4-3.3]
Tumor Necrosis Factor β (pg/mL) median (IQR)[†§]	35.0 [18.0-116.0]	20.5 [16.5-44.3]	36.5 [27.0-152.8]
Tumor necrosis factor receptor 2 (ng/mL) median (IQR)[#]	4.5 [3.6-5.7]	4.4 [3.5-5.8]	4.5 [3.7-5.6]

Table 1. *(continued)*

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Interferon γ (pg/mL) median (IQR)*§	5.1 [3.9-7.3]	4.8 [3.8-6.6]	5.7 [4.2-8.2]
Interleukin-6 (pg/mL) median (IQR)⁻	3.5 [2.2-5.8]	3.7 [2.5-6.8]	2.5 [2.1-4.1]
Interleukin-8 (pg/mL) median (IQR)#§	8.9 [6.8-12.0]	9.9 [7.1-12.6]	8.3 [6.5-10.3]
Interleukin-10 (pg/mL) median (IQR)#§	5.2 [3.6-9.4]	6.9 [4.1-15.0]	4.4 [3.0-6.0]
Interleukin-18 (pg/mL) median (IQR)*	171.0 [132.3-215.0]	173.0 [133.0-216.3]	169.5 [130.5-211.3]

*Measurable in all patients
#Measurable in >99% of patients, too low to detect in <1%
+Measurable in 76% of patients, too low to detect in 24%
⁻ Measurable in 38% of patients, too low to detect in 62%
†Measurable in 8% of patients, too low to detect in 92%
§ TNFβ, IFNγ, IL-10 and IL-18: total n= 473, ACS n=309, SAP n= 261

patients had at least 1 TCFA lesion, including 69 (12.1%) patients with at least 1 TCFA lesion with a plaque burden ≥ 70%. Concentrations of INFγ, TNF R2, IL-8, IL-10 and IL-18 were not normally distributed; these biomarkers were therefore ln-tranformed for further analyses. TNF-α, TNF-β and IL-6 were too low to detect in a large part of the patients, and thus were not examined as continuous variables in the statistical models. TNF-α was too low to detect in 24%, and hence was categorized into tertiles for further analyses. TNF-β and IL-6 were too low to detect in 92% and 62% of the patients, respectively, and these markers were dichotomized into measurable versus not measurable for further analyses. IL-10 concentrations could be measured in 99%. TNF R2, IL-8, IL-18 and IFNγ were measurable in all patients.

Biomarkers and extent of atherosclerosis

The results of the analyses for plaque burden of the entire measured segment are shown in Figure 1 and supplemental tables 1a,b and c. Higher TNF-α was associated with higher coronary plaque burden in patients with SAP (β [95%CI]: 4.45 [0.99-7.91], for the highest vs the lowest tertile of TNF-α). Such an effect could not be demonstrated in patients with ACS.

Furthermore, lower IL-10 concentrations were associated with higher coronary plaque burden in the full cohort (β [95%CI]: −3.88 [−6.00 – −1.76], for the highest vs the lowest tertile of IL-10). This effect was driven by both the SAP patients and the ACS patients. Although effect estimates for the highest tertile of IL-10 were similar in both groups (SAP: −2.95 [−6.23-0.33], ACS: −3.42 [−6.57 – −0.27], in the SAP patients the estimates, as well as the linear trend, did not reach statistical significance.

After multivariable adjustment, associations remained essentially the same for both TNF-α and IL-10.

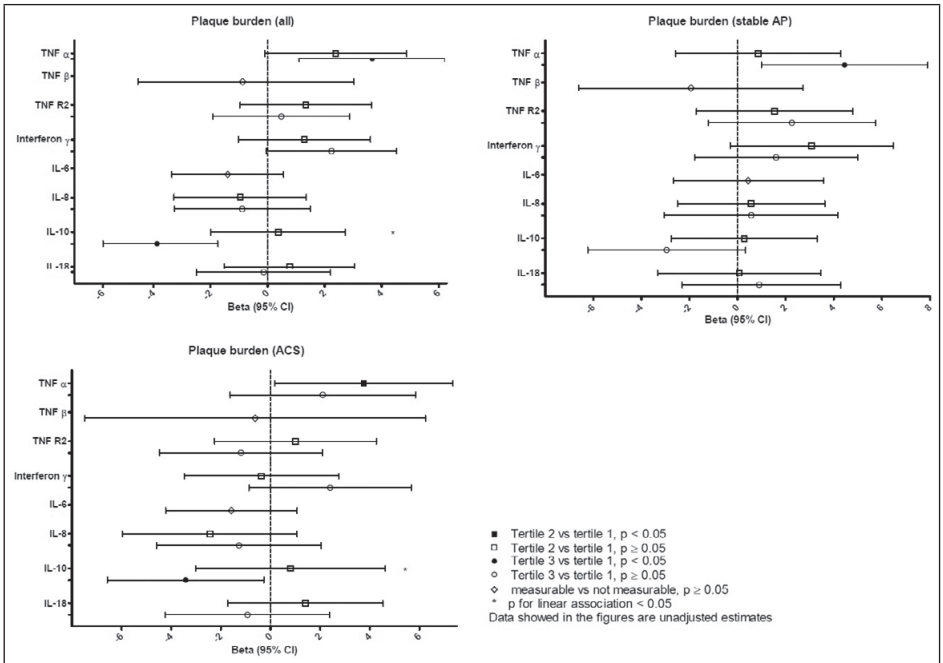

Figure 1. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in all patients, patients with stable AP and patients with ACS.

Biomarkers and composition of atherosclerosis

The results of the analyses for VH-TCFA lesions are displayed in Figure 2 and supplemental tables 2a, b and c. High TNF-α was positively associated with presence of VH-TCFA lesions in patients with SAP (OR[95%CI]: 2.30 [1.17-4.52] for the highest vs the lowest tertile of TNF-α). Such an effect was absent in patients with ACS. Furthermore, higher IL-8 seemed to confer lower risk of VH-TCFA in ACS patients; however, this effect was mainly driven by tertile 2. No associations were present between any of the other biomarkers and VH-TCFA.

Higher TNF-α was positively associated with presence of VH-TCFA lesions with a plaque burden ≥ 70% in the full cohort (OR[95%CI]: 2.85 [1.28-6.31] for the highest vs the lowest tertile of TNF-α) (table 4). This effect was driven by both patients with SAP and patients with ACS. Although the effect estimate reached statistical significance in the full cohort, this was not the case in the SAP and ACS groups. Nevertheless, the effect estimates for the highest tertile of TNF-α were similar in magnitude in both groups (SAP: 3.44 [0.89-13.29], ACS: 2.39 [0.89-6.45]. Higher IL-10 displayed an inverse association with presence of VH-TCFA lesions with a plaque burden ≥ 70% in the full cohort (OR[95%CI]: 0.31 [0.12-0.80] for the highest vs the lowest tertile of IL-10, p for trend=0.037). Again, effect estimates did not reach statistical significance in these separate groups.

After multivariable adjustment, associations remained essentially the same.

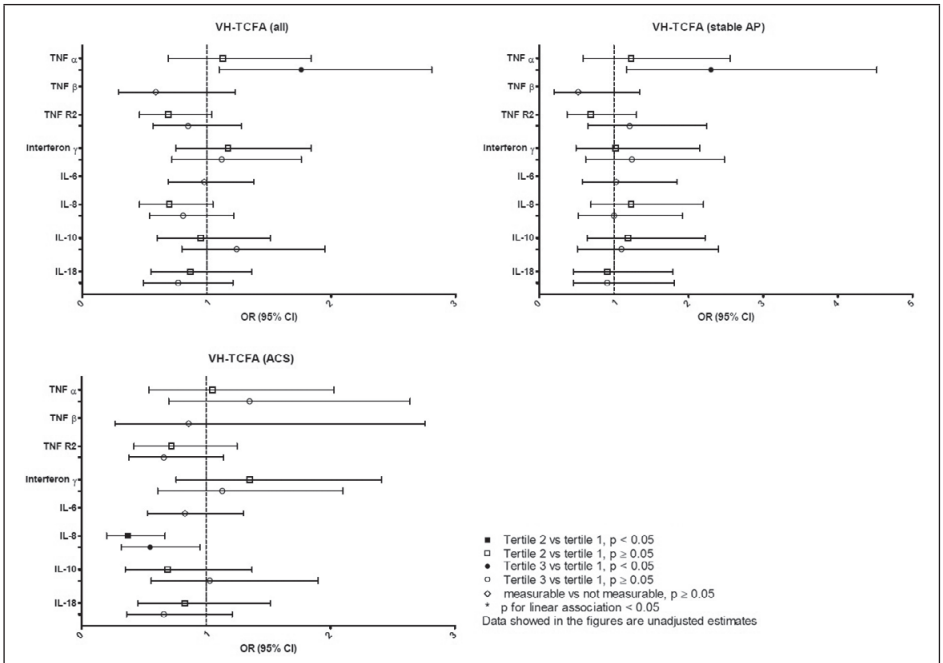

Figure 2. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in all patients, patients with stable AP and patients with ACS.

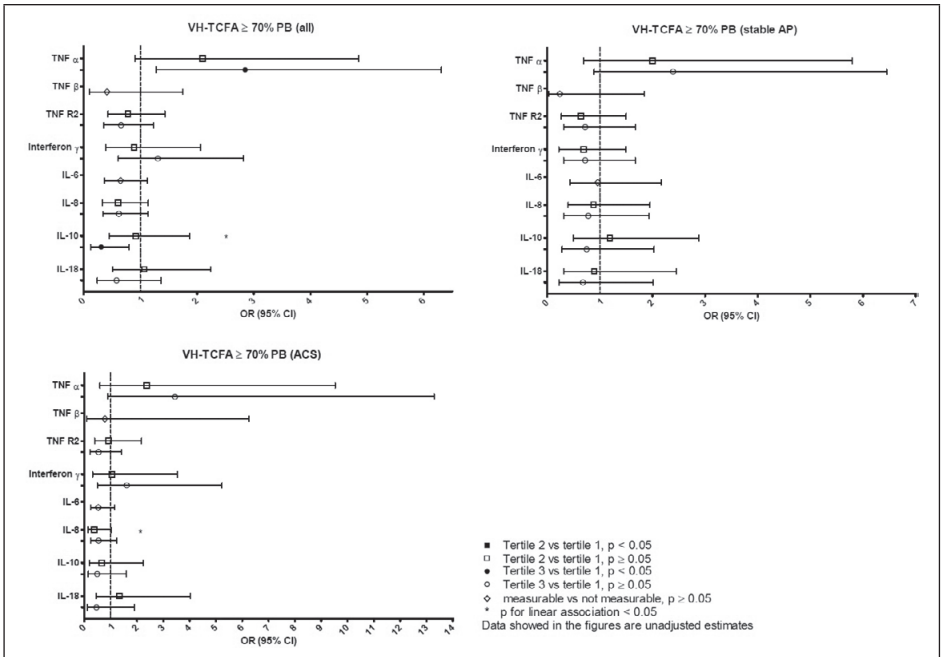

Figure 3. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in all patients, patients with stable AP and patients with ACS.

Biomarkers and MACE

Vital status was acquired for 569 (99.8%) patients. Response rate of the questionnaires that were sent to all living patients was 92.3%. After 1 year of follow-up, 56 patients reached the composite endpoint. Hazard ratios for the occurrence of MACE are shown in Figure 4 and supplemental tables 4a, b and c. Higher TNF R2 was associated with MACE in SAP patients (OR[95%CI]: 2.99 [1.10-8.13], per Ln (ng/mL) TNF R2) on univariable analysis; after multivariable adjustment, this association lost statistical significance. No significant associations could be demonstrated between any of the other biomarkers and MACE. Additional analysis of the composite of all-cause mortality or ACS (secondary endpoint) did not result in significant associations either.

DISCUSSION

This study examined whether circulating cytokine concentrations are associated with extent and composition of coronary atherosclerosis, as determined by IVUS and IVUS-VH in a non-culprit vessel, in patients with SAP or ACS undergoing coronary angiography. We also investigated whether these cytokines have prognostic value for cardiovascular

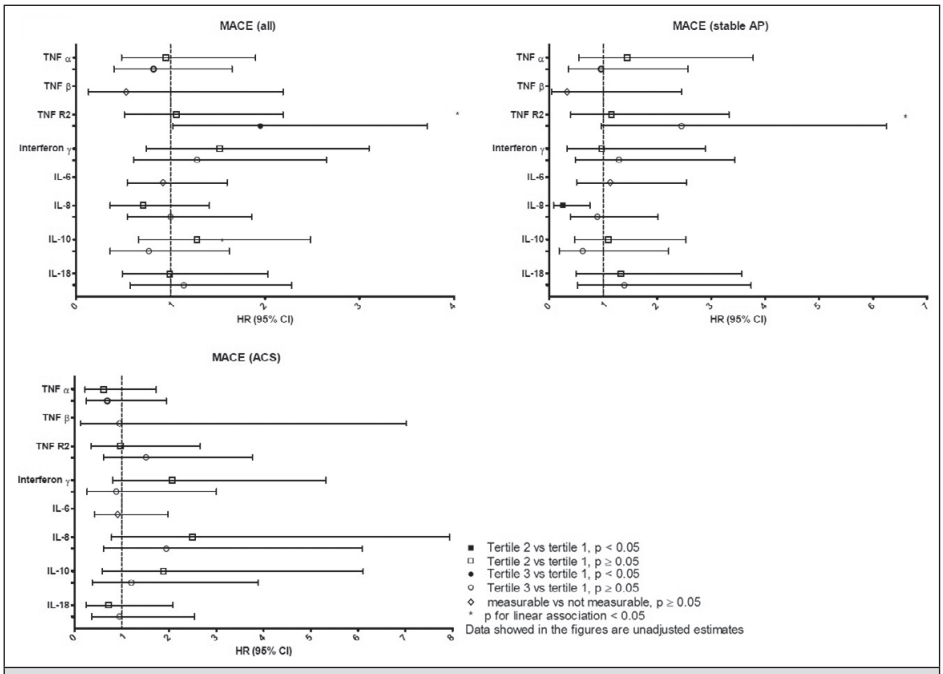

Figure 4. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in all patients, patients with stable AP and patients with ACS.

outcome. In patients with SAP, higher concentrations of TNF-α were associated with higher coronary plaque burden and with presence of VH-TCFA lesions, and displayed a tendency towards a positive association with presence of VH-TCFA lesion with a plaque burden ≥ 70%. Overall, higher concentrations of IL-10 were inversely associated with coronary plaque burden and with presence of VH-TCFA with a plaque burden ≥ 70%. These effects of IL-10 did not reach statistical significance in the separate groups. No associations were found between any of the studied cytokines and the occurrence of MACE.

Inflammation is known to play a major role in atherosclerosis. In a previous study in the current patient population, we have demonstrated an association between CRP and IVUS characteristics as well as incidence of MACE[15]. TNF-α is a proinflammatory cytokine that is secreted from activated innate immunity cells and is capable of inducing a cascade with a broad range of effects, including immunological activation, apoptosis, and procoagulative and antifibrinolytic actions, all of which can have an effect on the course of atherosclerosis [5, 16]. Experimental studies on the role of TNF-α in plaque development and stability in mice have rendered inconsistent results, some finding anti-atherogenic effects and others finding pro-atherogenic effects [5]. This discrepancy in results may be due to differences in underlying mechanisms of atherogenesis in different types of mouse models. A recent study [17] in human saphenous vein organ culture, to which a combination of TNF-α and LDL was applied, demonstrated phenotypic changes characteristic of the initial development of atherosclerotic plaques. Clinical studies on the role of TNF-α in cardiovascular disease have also rendered inconsistent results. A prior study found an increase of serum TNF-α in patients with MI and unstable angina pectoris compared to healthy subjects[18]. Ridker et al. [19] found that plasma concentrations of TNF-α are persistently elevated among post-MI patients at increased risk for recurrent coronary events. [20]. Furthermore, Naranjo et al. [21] found that TNF-α therapy was associated with a lower incidence of cardiovascular events in patients with rheumatoid arthritis, who are known to be at high cardiovascular risk. On the other hand, Cherneva et al. [22] and Sukhija et al. [23] examined the prognostic abilities of TNF-α in patients with known coronary artery disease, but did not find any associations between TNF-α and patient outcome. In the current study, we found that higher TNF-α level are associated with both extent of atherosclerosis and with plaque vulnerability in patients with SAP, which is in line with the presumed proinflammatory nature of this cytokine. On the other hand, we have recently demonstrated in the same study population [24] that presence of lesions with a high plaque burden, and presence of VH-TCFA lesions, are both independently associated with a higher MACE rate. However, higher TNF-α was not associated with the occurrence of MACE. Altogether, these findings imply that the deleterious effect of TNF-α does not translate into a higher MACE rate in the current study population. Possible explanations may include the fact that the magnitude of the

effect of TNF-α is small in the context of this multifactorial disease, or that the current study lacks statistical power to expose such an effect.

IL-10 is an anti-inflammatory cytokine that is produced by macrophages and lymphocytes [6]. This cytokine is capable of inhibiting many cellular processes that may play an important role in atherosclerotic lesion development and in the modulation of plaque composition [6, 25]. Mallat et al. [25] investigated atherosclerotic lesions in IL-10 deficient mice and showed increased infiltration of inflammatory cells, increased production of INF-γ, and decreased collagen content, which resulted in development of atheromatous lesions with signs of increased vulnerability. Several clinical studies have been performed on IL-10 and cardiovascular disease. Heeschen et al. [26] demonstrated that a reduced serum IL-10 level in patients with ACS is indicative of a poor prognosis. Most subsequent studies on the association of elevated circulating IL-10 levels with cardiovascular outcome have demonstrated positive associations with better prognosis [27-31]. In line with this, we found an inverse association between IL-10 and coronary plaque burden as well as between IL-10 and presence of large, vulnerable plaques (i.e., VH-TCFA lesions with a plaque burden ≥ 70%) in the overall study population. However, we did not find an association of IL-10 with presence of TCFA lesions in general. These results suggest that IL-10 may in particular be associated with lower extent of coronary atherosclerosis and slower growth of VH-TCFAs. In any case, these findings further support the hypothesis of a protective role of IL-10 in atherosclerosis. In a recent study performed in the same population[24], we have demonstrated that lesions with a high plaque burden, as well as VH-TCFA lesions with a plaque burden of ≥70%, are both independently associated with a higher MACE rate. While an inverse association was present of IL-10 with both plaque burden and with presence of VH-TCFA lesions with plaque burden >70% in the current study, an inverse association between IL-10 and MACE could not be demonstrated. Taken together, these results imply that the potential advantageous effect of IL-10 on plaque burden and large TCFA does not translate into a lower MACE rate. Again, the magnitude of the effect of IL-10 may be small, or statistical power may be insufficient to demonstrate the effect.

Since no associations could be demonstrated between the individual cytokines and MACE, clinical usefulness of this study may be debated. Nevertheless, we believe that our findings are informative, because they provide additional insights into the complex pathophysiologic relation between cytokines and cardiovascular disease. Moreover, we did not find any associations between several cytokines we examined and the extent or composition of atherosclerosis. Analysis of some of the biomarkers (TNF-β and IL-6) was complicated by the fact that over 50% of the measurements were too low to detect. Cytokine assays are generally known to display limitations in terms of % detectability [32, 33]. This makes clinical investigations into the pathophysiological role and the prognostic value of these biomarkers challenging. In line with this, few clinical studies have

been performed on circulating TNF-β. Furthermore, IL-6 is known to have large circadian variations, and a relatively short half-life of less than 6 hours [34] which also makes this marker difficult to investigate. Clinical studies on circulating TNFR2, INFγ, and IL-8 in patients with coronary artery disease are also limited in number. IL-18 has been examined more often, and has been suggested to be associated with the presence and severity of coronary atherosclerosis [35, 36]. In the present study, we could not demonstrate such an association.

Some aspects of this study warrant consideration. Our study population consisted of patients with SAP as well as patients with ACS. The group of patients with ACS is likely to be more heterogeneous, which may have influenced the findings. To account for this, we have performed the analyses separately in both groups. Furthermore, VH-IVUS imaging took place of a prespecified single target segment of a single non-culprit coronary artery, based on the assumption that such a non-stenotic segment adequately reflects coronary wall pathophysiology of the larger coronary tree. Although this assumption may be debated, previous studies evaluating IVUS have demonstrated that the coronary wall of comparable non-culprit, non-stenotic segments of a single vessel does reflect coronary disease burden at large and is associated with subsequent cardiovascular outcome [24, 37, 38]. Moreover, it is important to note that IVUS is formally not capable of detecting the most rupture prone of all plaque phenotypes, the TCFA [39, 40], because the spatial resolution of IVUS is insufficient for thin cap detection (23, 24). Nonetheless, a concept of VH-IVUS derived TCFA has been postulated for plaques with a plaque burden ≥ 40% and a confluent necrotic core ≥ 10% in direct contact with the lumen in at least three VH-IVUS frames (13, 23). Notably, we have recently demonstrated that such VH-IVUS derived TCFA lesions are strongly and independently predictive of the occurrence of major adverse cardiac events within the current study population [24].

In conclusion, in patients undergoing coronary angiography, higher circulating TNF-α was associated with higher plaque burden and with presence of VH-TCFA lesions in patients with SAP. Overall, lower circulating IL-10 was associated with higher plaque burden and with presence of VH-TCFA lesions with a plaque burden ≥ 70%. The latter effects did not reach statistical significance in the separate SAP and ACS groups. These cytokines were not associated with occurrence of MACE. These in-vivo findings illustrate that TNF-α and IL-10 appear to play a role in both extent and vulnerability of coronary atherosclerosis, which is in line with experimental studies. However, their clinical value in terms of risk stratification warrants further investigation.

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SUPPLEMENTAL TABLES

Supplemental table 1a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in all patients.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	2.39 (−0.10-4.88)	0.060	1.94 (−0.52-4.39)	0.12
Tertile 3	3.67 (1.10-6.23)	0.005	3.13 (0.63-5.62)	0.014
TNFbeta				
not measurable	reference		reference	
measurable	−0.88 (−4.78-3.03)	0.66	−1.39 (−5.25- −2.47)	0.48
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.34 (−0.96-3.64)	0.25	−0.56 (−2.89-1.76)	0.63
Tertile 3	0.48 (−1.92-2.88)	0.69	−1.73 (−4.29-0.82)	0.18
Ln (TNFR2)	0.61 (−1.98-3.20)	0.65	−2.43 (−5.15-0.29)	0.080
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.29 (−1.02-3.61)	0.27	0.41 (−1.86-2.67)	0.73
Tertile 3	2.24 (−0.05-4.53)	0.055	0.51 (−1.98-2.99)	0.69
Ln (Interferon γ)	1.61 (−0.15-3.37)	0.072	0.11 (−1.71-1.92)	0.91
IL-6				
not measurable	reference		reference	
measurable	−1.40 (−3.36-0.56)	0.16	−0.70 (−2.74-1.35)	0.50
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−0.96 (−3.29-1.36)	0.42	−0.78 (−3.06-1.49)	0.50
Tertile 3	−0.89 (−3.27-1.50)	0.46	−1.63 (−4.08-0.82)	0.19
Ln (IL8)	−0.07 (−2.22-2.09)	0.95	−0.54 (−2.70-1.62)	0.63
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.37 (−2.00-2.73)	0.76	0.63 (−1.73-3.00)	0.60
Tertile 3	−3.88 (−6.00- −1.76)	<0.001	−3.27 (−5.55- −0.99)	0.005
Ln (IL10)	−1.52 (−2.49- −0.55)	0.002	−1.25 (−2.26- −0.24)	0.016
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.77 (−1.52-3.06)	0.51	1.04 (−1.24-3.33)	0.37
Tertile 3	−0.14 (−2.50-2.21)	0.91	0.14 (−2.15-2.42)	0.91
Ln (IL18)	−0.84 (−3.17-1.48)	0.48	−0.53 (−2.80-1.74)	0.65

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 1b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in patients with stable AP.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.86 (−2.58-4.30)	0.62	0.33 (−3.09-3.74)	0.85
Tertile 3	4.45 (0.99-7.91)	0.012	4.64 (1.11-8.16)	0.010
TNFbeta				
not measurable	reference		reference	
measurable	−1.94 (−6.61-2.73)	0.41	−1.63 (−6.27-3.00)	0.49
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.54 (−1.71-4.80)	0.35	−0.16 (−3.49-3.18)	0.93
Tertile 3	2.26 (−1.22-5.73)	0.20	0.40 (−3.48-4.29)	0.84
Ln (TNFR2)	2.90 (−0.94-6.74)	0.14	0.64 (−3.54-4.82)	0.76
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	3.08 (−0.31-6.47)	0.075	2.57 (−0.92-6.05)	0.15
Tertile 3	1.60 (−1.79-4.99)	0.35	0.40 (−3.22-4.02)	0.83
Ln (Interferon γ)	1.39 (−1.01-3.80)	0.26	0.44 (−2.07-2.95)	0.73
IL-6				
not measurable	reference		reference	
measurable	0.44 (−2.68-3.57)	0.78	0.47 (−2.76-3.70)	0.78
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.56 (−2.50-3.63)	0.72	0.17 (−2.90-3.23)	0.91
Tertile 3	0.57 (−3.04-4.17)	0.76	−0.18 (−3.87-3.50)	0.92
Ln (IL8)	2.03 (−1.11-5.16)	0.21	1.10 (−2.08-4.28)	0.50
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.28 (−2.76-3.32)	0.86	0.34 (−2.65-3.33)	0.82
Tertile 3	−2.95 (−6.23-0.33)	0.078	−3.30 (−6.64-0.04)	0.053
Ln (IL10)	−1.03 (−3.02-0.95)	0.31	−1.34 (−3.34-0.66)	0.19
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.07 (−3.33-3.47)	0.97	−0.34 (−3.71-3.02)	0.84
Tertile 3	0.99 (−2.30-4.29)	0.55	0.11 (−3.24-3.47)	0.95
Ln (IL18)	1.72 (−1.83-5.28)	0.34	0.99 (−2.57-4.56)	0.58

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 1c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in patients with ACS.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	3.76 (0.17-7.35)	0.040	2.98 (−0.65-6.61)	0.11
Tertile 3	2.10 (−1.63-5.84)	0.27	1.79 (−1.83-5.41)	0.33
TNFbeta				
not measurable	reference		reference	
measurable	−0.62 (−7.48-6.24)	0.86	−1.08 (−7.98-5.82)	0.76
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.01 (−2.26-4.27)	0.54	−1.19 (−4.48-2.10)	0.48
Tertile 3	−1.19 (−4.47-2.09)	0.48	−3.37 (−6.86-0.13)	0.059
Ln (TNFR2)	−1.18 (−4.67-2.30)	0.51	−4.53 (−8.17- −0.89)	0.015
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−0.37 (−3.47-2.74)	0.82	−0.96 (−4.01-2.08)	0.53
Tertile 3	2.40 (−0.87-5.67)	0.15	0.58 (−2.89-4.05)	0.74
Ln (Interferon γ)	1.09 (−1.51-3.70)	0.41	−0.22 (−2.89-2.46)	0.87
IL-6				
not measurable	reference		reference	
measurable	−1.58 (−4.22-1.07)	0.24	−1.49 (−4.18-1.20)	0.28
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−2.44 (−5.96-1.07)	0.17	−2.25 (−5.71-1.22)	0.20
Tertile 3	−1.27 (−4.58-2.03)	0.45	−2.77 (−6.12-0.59)	0.11
Ln (IL8)	−0.99 (−3.99-2.02)	0.52	−2.02 (−5.02-0.97)	0.19
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.81 (−3.01-4.63)	0.68	1.31 (−2.65-5.28)	0.51
Tertile 3	−3.42 (−6.57- −0.27)	0.034	−3.12 (−6.24-0.01)	0.051
Ln (IL10)	−1.30 (−2.52- −0.08)	0.038	−1.27 (−2.48- −0.05)	0.041
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.40 (−1.72-4.52)	0.38	2.11 (−1.14-5.35)	0.20
Tertile 3	−0.93 (−4.23-2.37)	0.58	0.07 (−3.21-3.34)	0.97
Ln (IL18)	−2.30 (−5.35-0.75)	0.14	−1.52 (−4.55-1.51)	0.32

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in all patients.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.13 (0.69-1.84)	0.63	1.12 (0.68-1.83)	0.67
Tertile 3	1.76 (1.10-2.81)	0.018	1.82 (1.13-2.93)	0.014
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.59 (0.29-1.23)	0.16	0.70 (0.33-1.47)	0.34
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.46-1.04)	0.079	0.68 (0.44-1.04)	0.078
Tertile 3	0.85 (0.57-1.28)	0.45	0.84 (0.54-1.30)	0.43
LN (TNFR2)	0.87 (0.55-1.37)	0.55	0.85 (0.52-1.40)	0.52
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.17 (0.75-1.84)	0.50	1.21 (0.76-1.91)	0.42
Tertile 3	1.12 (0.72-1.76)	0.62	1.22 (0.75-1.97)	0.43
LN (Interferon γ)	1.08 (0.76-1.52)	0.68	1.15 (0.79-1.66)	0.47
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.98 (0.69-1.38)	0.90	0.97 (0.67-1.41)	0.87
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.70 (0.46-1.05)	0.085	0.69 (0.46-1.06)	0.089
Tertile 3	0.81 (0.54-1.22)	0.81	0.77 (0.50-1.18)	0.23
LN (IL8)	0.91 (0.62-1.33)	0.63	0.87 (0.59-1.30)	0.50
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.95 (0.60-1.51)	0.84	0.95 (0.59-1.52)	0.83
Tertile 3	1.24 (0.80-1.95)	0.34	1.21 (0.75-1.94)	0.44
LN (IL10)	1.15 (0.95-1.39)	0.16	1.13 (0.92-1.39)	0.25
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.87 (0.55-1.36)	0.54	0.90 (0.57-1.43)	0.66
Tertile 3	0.77 (0.49-1.21)	0.25	0.76 (0.48-1.20)	0.24
LN (IL18)	0.90 (0.57-1.42)	0.64	0.91 (0.57-1.44)	0.67

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in patients with stable AP.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.23 (0.59-2.56)	0.58	1.27 (0.60-2.66)	0.53
Tertile 3	2.30 (1.17-4.52)	0.015	2.31 (1.16-4.59)	0.017
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.52 (0.20-1.35)	0.18	0.52 (0.20-1.37)	0.19
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.37-1.30)	0.25	0.67 (0.35-1.29)	0.23
Tertile 3	1.21 (0.65-2.24)	0.55	1.14 (0.58-2.23)	0.71
LN (TNFR2)	1.44 (0.70-2.94)	0.32	1.38 (0.62-3.04)	0.43
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.02 (0.49-2.15)	0.95	0.96 (0.45-2.05)	0.91
Tertile 3	1.24 (0.62-2.48)	0.55	1.19 (0.57-2.50)	0.64
LN (Interferon γ)	1.23 (0.74-2.05)	0.43	1.23 (0.71-2.13)	0.45
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	1.03 (0.58-1.84)	0.92	0.95 (0.51-1.76)	0.87
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.23 (0.69-2.20)	0.48	1.27 (0.70-2.29)	0.44
Tertile 3	1.00 (0.52-1.92)	1.00	0.95 (0.48-1.85)	0.87
LN (IL8)	1.15 (0.64-2.05)	0.64	1.08 (0.59-1.97)	0.81
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.19 (0.64-2.22)	0.58	1.22 (0.65-2.30)	0.54
Tertile 3	1.10 (0.51-2.40)	0.81	1.06 (0.47-2.36)	0.90
LN (IL10)	1.41 (0.93-2.15)	0.11	1.39 (0.90-2.14)	0.14
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.91 (0.46-1.79)	0.78	0.88 (0.44-1.76)	0.72
Tertile 3	0.91 (0.46-1.81)	0.78	0.82 (0.41-1.68)	0.60
LN (IL18)	1.01 (0.48-2.13)	0.99	0.95 (0.44-2.04)	0.89

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in patients with ACS.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.05 (0.54-2.03)	0.89	0.89 (0.45-1.78)	0.74
Tertile 3	1.35 (0.70-2.64)	0.37	1.43 (0.72-2.84)	0.31
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.86 (0.27-2.76)	0.80	0.98 (0.29-3.34)	0.98
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.72 (0.42-1.25)	0.25	0.69 (0.39-1.22)	0.20
Tertile 3	0.66 (0.38-1.14)	0.14	0.62 (0.34-1.12)	0.11
LN (TNFR2)	0.63 (0.34-1.14)	0.13	0.59 (0.30-1.15)	0.12
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.35 (0.76-2.41)	0.30	1.38 (0.77-2.49)	0.28
Tertile 3	1.13 (0.61-2.10)	0.69	1.15 (0.60-2.21)	0.68
LN (Interferon γ)	1.06 (0.65-1.73)	0.83	1.05 (0.63-1.76)	0.86
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.83 (0.53-1.30)	0.42	0.96 (0.59-1.55)	0.86
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.37 (0.20-0.67)	0.001	0.40 (0.21-0.74)	0.004
Tertile 3	0.55 (0.32-0.95)	0.033	0.60 (0.33-1.08)	0.086
LN (IL8)	0.70 (0.42-1.17)	0.17	0.76 (0.44-1.30)	0.31
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.35-1.37)	0.29	0.76 (0.37-1.54)	0.44
Tertile 3	1.03 (0.56-1.90)	0.93	1.14 (0.61-2.14)	0.68
LN (IL10)	1.02 (0.81-1.28)	0.90	1.03 (0.82-1.31)	0.79
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.83 (0.45-1.52)	0.55	0.90 (0.48-1.69)	0.75
Tertile 3	0.66 (0.36-1.21)	0.18	0.65 (0.35-1.21)	0.17
LN (IL18)	0.82 (0.46-1.46)	0.50	0.82 (0.46-1.49)	0.52

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in all patients.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.10 (0.91-4.85)	0.083	2.11 (0.91-4.93)	0.084
Tertile 3	2.85 (1.28-6.31)	0.01	2.78 (1.24-6.23)	0.013
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.41 (0.10-1.75)	0.23	0.41 (0.10-1.78)	0.24
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.78 (0.43-1.43)	0.42	0.67 (0.36-1.25)	0.20
Tertile 3	0.66 (0.35-1.23)	0.19	0.52 (0.26-1.04)	0.064
LN (TNFR2)	0.65 (0.32-1.30)	0.22	0.50 (0.23-1.09)	0.081
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.89 (0.39-2.06)	0.79	0.78 (0.34-1.83)	0.57
Tertile 3	1.31 (0.61-2.82)	0.49	0.93 (0.41-2.14)	0.87
LN (Interferon γ)	1.21 (0.66-2.21)	0.54	0.93 (0.48-1.80)	0.83
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.65 (0.37-1.12)	0.12	0.75 (0.42-1.36)	0.35
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.61 (0.33-1.14)	0.12	0.63 (0.34-1.18)	0.15
Tertile 3	0.62 (0.34-1.14)	0.12	0.64 (0.34-1.22)	0.17
LN (IL8)	0.57 (0.32-1.02)	0.059	0.57 (0.31-1.05)	0.069
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.92 (0.45-1.87)	0.81	0.97 (0.47-2.02)	0.94
Tertile 3	0.31 (0.12-0.80)	0.016	0.36 (0.13-0.97)	0.043
LN (IL10)	0.64 (0.42-0.97)	0.037	0.69 (0.44-1.08)	0.10
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.07 (0.51-2.24)	0.87	1.09 (0.51-2.32)	0.82
Tertile 3	0.58 (0.24-1.36)	0.21	0.59 (0.25-1.40)	0.23
LN (IL18)	0.49 (0.23-1.08)	0.077	0.51 (0.22-1.14)	0.10

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in patients with stable AP.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.00 (0.69-5.79)	0.20	2.11 (0.72-6.18)	0.17
Tertile 3	2.39 (0.89-6.45)	0.086	2.48 (0.90-6.79)	0.078
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.24 (0.03-1.85)	0.17	0.24 (0.03-1.86)	0.17
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.64 (0.27-1.50)	0.30	0.63 (0.26-1.53)	0.31
Tertile 3	0.72 (0.31-1.67)	0.45	0.71 (0.28-1.77)	0.46
LN (TNFR2)	0.79 (0.29-2.15)	0.65	0.81 (0.27-2.42)	0.70
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.22-2.19)	0.53	0.64 (0.20-2.05)	0.45
Tertile 3	0.90 (0.32-2.53)	0.85	0.83 (0.28-2.47)	0.73
LN (Interferon γ)	0.96 (0.44-2.09)	0.91	0.90 (0.38-2.12)	0.81
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.96 (0.43-2.17)	0.93	0.99 (0.42-2.33)	0.99
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.88 (0.40-1.95)	0.76	0.91 (0.41-2.04)	0.82
Tertile 3	0.78 (0.31-1.94)	0.59	0.79 (0.31-2.00)	0.62
LN (IL8)	0.85 (0.38-1.94)	0.70	0.87 (0.37-2.01)	0.74
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.19 (0.50-2.88)	0.69	1.23 (0.50-2.98)	0.66
Tertile 3	0.75 (0.28-2.03)	0.57	0.74 (0.27-2.05)	0.57
LN (IL10)	0.66 (0.32-1.36)	0.26	0.64 (0.30-1.36)	0.25
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.89 (0.32-2.45)	0.82	0.87 (0.31-2.42)	0.79
Tertile 3	0.68 (0.23-2.01)	0.48	0.63 (0.21-1.93)	0.42
LN (IL18)	0.55 (0.18-1.71)	0.30	0.53 (0.17-1.68)	0.28

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in patients with ACS.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.37 (0.59-9.53)	0.23	2.07 (0.50-8.65)	0.32
Tertile 3	3.44 (0.89-13.29)	0.073	3.57 (0.90-14.13)	0.070
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.78 (0.10-6.25)	0.82	0.86 (0.10-7.15)	0.89
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.92 (0.39-2.17)	0.86	0.66 (0.27-1.66)	0.38
Tertile 3	0.54 (0.21-1.42)	0.22	0.35 (0.12-1.03)	0.056
LN (TNFR2)	0.51 (0.19-1.39)	0.19	0.30 (0.09-0.97)	0.044
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.04 (0.31-3.54)	0.95	1.02 (0.29-3.56)	0.98
Tertile 3	1.61 (0.50-5.22)	0.43	1.12 (0.32-3.86)	0.86
LN (Interferon γ)	1.40 (0.53-3.71)	0.50	1.02 (0.37-2.84)	0.97
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.53 (0.25-1.14)	0.10	0.60 (0.27-1.36)	0.22
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.37 (0.14-1.01)	0.052	0.38 (0.13-1.07)	0.066
Tertile 3	0.54 (0.24-1.23)	0.14	0.50 (0.20-1.23)	0.13
LN (IL8)	0.42 (0.18-0.96)	0.039	0.38 (0.16-0.91)	0.029
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.65 (0.19-2.23)	0.49	0.69 (0.19-2.50)	0.57
Tertile 3	0.49 (0.15-1.59)	0.24	0.53 (0.16-1.79)	0.31
LN (IL10)	0.69 (0.40-1.20)	0.19	0.71 (0.41-1.23)	0.22
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.33 (0.44-4.02)	0.61	1.37 (0.43-4.42)	0.60
Tertile 3	0.46 (0.11-1.90)	0.28	0.52 (0.12-2.21)	0.37
LN (IL18)	0.44 (0.14-1.35)	0.15	0.45 (0.13-1.54)	0.20

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 4a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in all patients.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.95 (0.48-1.90)	0.89	0.88 (0.44-1.77)	0.73	0.96 (0.48-1.93)	0.91
Tertile 3	0.82 (0.40-1.65)	0.57	0.76 (0.37-1.54)	0.44	0.74 (0.36-1.51)	0.40
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.53 (0.13-2.19)	0.38	0.51 (0.12-2.08)	0.34	0.54 (0.13-2.23)	0.40
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.06 (0.51-2.19)	0.88	0.88 (0.42-1.86)	0.75	1.01 (0.48-2.09)	0.99
Tertile 3	1.95 (1.02-3.72)	0.042	1.55 (0.77-3.09)	0.22	1.71 (0.88-3.32)	0.11
LN (TNFR2)	2.34 (1.20-4.55)	0.012	1.92 (0.92-3.99)	0.08	1.81 (0.91-3.57)	0.090
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.52 (0.74-3.10)	0.25	1.38 (0.68-2.84)	0.38	1.47 (0.72-3.01)	0.29
Tertile 3	1.28 (0.61-2.65)	0.51	0.97 (0.45-2.09)	0.94	1.15 (0.55-2.42)	0.72
LN (Interferon γ)	1.15 (0.67-1.98)	0.62	0.93 (0.52-1.65)	0.79	1.08 (0.63-1.87)	0.78
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.923 (0.54-1.60)	0.79	1.03 (0.58-1.81)	0.93	0.78 (0.43-1.40)	0.40
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.71 (0.36-1.41)	0.33	0.71 (0.36-1.40)	0.32	0.66 (0.33-1.32)	0.24
Tertile 3	1.00 (0.54-1.86)	1.00	0.95 (0.50-1.80)	0.87	0.83 (0.43-1.58)	0.56
LN (IL8)	1.25 (0.69-2.27)	0.47	1.18 (0.64-2.17)	0.60	1.07 (0.58-1.97)	0.84
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.28 (0.66-2.48)	0.47	1.31 (0.67-2.57)	0.43	1.12 (0.57-2.20)	0.75
Tertile 3	0.77 (0.36-1.62)	0.48	0.83 (0.38-1.81)	0.65	0.74 (0.35-1.57)	0.43
LN (IL10)	0.98 (0.72-1.32)	0.88	1.03 (0.75-1.42)	0.87	0.98 (0.71-1.34)	0.89
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.99 (0.49-2.03)	0.98	0.98 (0.48-2.02)	0.96	1.10 (0.53-2.27)	0.81
Tertile 3	1.14 (0.57-2.28)	0.71	1.18 (0.59-2.36)	0.65	1.18 (0.58-2.37)	0.65
LN (IL18)	1.10 (0.54-2.21)	0.80	1.15 (0.56-2.36)	0.71	1.05 (0.53-2.06)	0.89

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

#additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.

Supplemental table 4b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in patients with stable AP.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.44 (0.55-3.78)	0.46	1.40 (0.53-3.70)	0.50	1.45 (0.55-3.83)	0.46
Tertile 3	0.96 (0.36-2.57)	0.93	0.95 (0.35-2.55)	0.91	0.81 (0.29-2.24)	0.68
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.33 (0.05-2.46)	0.28	0.35 (0.05-2.55)	0.30	0.34 (0.05-2.47)	0.28
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.15 (0.40-3.33)	0.79	1.09 (0.37-3.18)	0.88	1.08 (0.37-3.11)	0.89
Tertile 3	2.45 (0.96-6.25)	0.062	2.38 (0.88-6.46)	0.087	2.07 (0.78-5.44)	0.14
LN (TNFR2)	2.99 (1.10-8.13)	0.031	2.80 (0.97-8.07)	0.057	2.29 (0.80-6.53)	0.12
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.97 (0.33-2.89)	0.96	0.93 (0.31-2.76)	0.89	0.94 (0.31-2.82)	0.91
Tertile 3	1.29 (0.48-3.44)	0.61	1.13 (0.41-3.15)	0.82	1.17 (0.43-3.16)	0.76
LN (Interferon γ)	1.41 (0.68-2.91)	0.36	1.26 (0.59-2.69)	0.56	1.30 (0.62-2.71)	0.49
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	1.13 (0.51-2.55)	0.76	1.19 (0.53-2.68)	0.67	0.87 (0.36-2.10)	0.76
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.25 (0.08-0.75)	0.014	0.25 (0.08-0.74)	0.012	0.23 (0.07-0.69)	0.009
Tertile 3	0.89 (0.39-2.01)	0.78	0.87 (0.38-1.96)	0.73	0.71 (0.30-1.68)	0.44
LN (IL8)	1.03 (0.44-2.41)	0.94	0.98 (0.42-2.28)	0.95	0.81 (0.34-1.97)	0.65
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.09 (0.47-2.53)	0.83	1.11 (0.48-2.57)	0.81	1.08 (0.47-2.51)	0.85
Tertile 3	0.62 (0.18-2.21)	0.47	0.60 (0.17-2.12)	0.42	0.50 (0.13-1.90)	0.31
LN (IL10)	1.28 (0.73-2.27)	0.39	1.26 (0.71-2.22)	0.43	1.17 (0.65-2.14)	0.60
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.33 (0.50-3.57)	0.57	1.32 (0.49-3.55)	0.58	1.20 (0.44-3.27)	0.72
Tertile 3	1.39 (0.52-3.74)	0.51	1.32 (0.49-3.55)	0.58	1.15 (0.42-3.18)	0.78
LN (IL18)	1.78 (0.61-5.19)	0.29	1.70 (0.58-5.02)	0.33	1.49 (0.50-4.44)	0.48

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.

Supplemental table 4c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in patients with ACS.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.61 (0.22-1.72)	0.35	0.55 (0.20-1.55)	0.26	0.64 (0.22-1.83)	0.40
Tertile 3	0.69 (0.25-1.94)	0.48	0.61 (0.22-1.73)	0.35	0.62 (0.22-1.79)	0.38
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.95 (0.13-7.02)	0.96	0.96 (0.13-7.09)	0.97	1.01 (0.14-7.52)	0.99
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.97 (0.35-2.66)	0.95	0.74 (0.26-2.10)	0.57	0.93 (0.33-2.59)	0.89
Tertile 3	1.51 (0.61-3.76)	0.37	1.01 (0.37-2.72)	0.99	1.27 (0.49-3.31)	0.63
LN (TNFR2)	1.95 (0.77-4.96)	0.16	1.39 (0.48-4.00)	0.54	1.41 (0.53-3.74)	0.49
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	2.06 (0.80-5.32)	0.13	1.92 (0.74-4.97)	0.18	1.90 (0.73-4.94)	0.19
Tertile 3	0.88 (0.26-3.00)	0.83	0.68 (0.19-2.37)	0.54	0.77 (0.22-2.73)	0.69
LN (Interferon γ)	0.80 (0.35-1.83)	0.60	0.65 (0.28-1.51)	0.32	0.75 (0.32-1.78)	0.52
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.91 (0.42-1.97)	0.82	0.91 (0.42-1.97)	0.81	0.73 (0.32-1.70)	0.47
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	2.49 (0.78-7.94)	0.12	2.36 (0.74-7.58)	0.15	2.37 (0.74-7.59)	0.15
Tertile 3	1.94 (0.62-6.09)	0.26	1.48 (0.46-4.80)	0.51	1.56 (0.48-5.07)	0.46
LN (IL8)	1.70 (0.71-4.06)	0.23	1.38 (0.56-3.41)	0.49	1.43 (0.58-3.51)	0.43
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.88 (0.58-6.11)	0.29	1.81 (0.55-5.97)	0.33	1.51 (0.45-5.04)	0.51
Tertile 3	1.20 (0.37-3.89)	0.76	1.20 (0.37-3.90)	0.77	1.10 (0.34-3.60)	0.88
LN (IL10)	0.95 (0.63-1.42)	0.80	0.96 (0.64-1.43)	0.84	0.95 (0.63-1.44)	0.81
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.72 (0.25-2.08)	0.55	0.67 (0.23-1.96)	0.47	0.94 (0.31-2.81)	0.91
Tertile 3	0.95 (0.36-2.54)	0.92	1.04 (0.39-2.77)	0.94	1.09 (0.40-2.96)	0.87
LN (IL18)	0.77 (0.29-2.00)	0.58	0.82 (0.30-2.23)	0.70	0.76 (0.31-1.89)	0.56

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

#additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.

CORONARY VULNERABILITY

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VON WILLEBRAND FACTOR IN RELATION TO CORONARY PLAQUE CHARACTERISTICS AND CARDIOVASCULAR OUTCOME

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ABSTRACT

Objective: High VWF plasma levels are associated with an increased risk of coronary artery disease. It has been suggested that the increase of VWF levels is partly due to endothelial dysfunction and atherosclerosis. Our aim was to investigate the association between coronary plaque burden, the presence of high-risk coronary lesions as measured by intravascular ultrasound virtual histology (IVUS-VH) and VWF levels. In addition, we studied the association between VWF levels and 1-year cardiovascular outcome.

Methods: Between 2008 and 2011, IVUS-VH imaging of a non-culprit coronary artery was performed in 581 patients undergoing coronary angiography for acute coronary syndrome (ACS) (n= 318) or stable angina pectoris (SAP) (n= 263). Arterial blood was sampled prior to the coronary angiography. VWF antigen (VWF:Ag) levels were measured using ELISA (n= 577).

Results: Patients with ACS had significantly higher VWF:Ag levels than SAP patients (median 1.73 IU/ml [IQR 1.27-2.31] vs. 1.26 IU/ml [0.93-1.63], $p<0.001$). High coronary plaque burden was associated with higher VWF:Ag levels ($\beta= 0.12$, $p=0.027$) in SAP patients, but not in ACS patients. In ACS patients, VWF:Ag levels were associated with 1-year MACE (HR 4.14 per SD increase of lnVWF:Ag, 95% CI 1.47-11.6), whereas in SAP patients VWF:Ag levels predicted 1-year all-cause death and hospitalisation for ACS (HR 7.07 95% CI 1.40-35.6).

Conclusions: Coronary plaque burden was associated with VWF:Ag levels in SAP patients undergoing coronary angiography. In ACS and SAP patients, high VWF levels are predictive of adverse cardiovascular outcome and death during 1-year follow-up.

INTRODUCTION

Von Willebrand Factor (VWF) is a multimeric protein that plays a crucial role in primary hemostasis by mediating platelet adhesion and aggregation (1). VWF is produced by endothelial cells and megakaryocytes and stored in Weibel-Palade bodies in the endothelium and alpha-granules of platelets. VWF plasma levels are increased at moments of endothelial damage and are a marker of endothelial dysfunction (2).

It is well known that high VWF levels are associated with an increased risk of coronary heart disease and ischemic stroke in the general population (3-8). However, the underlying mechanisms of this association are still unclear. As high VWF levels are seen in situations with endothelial dysfunction, which is an important early process in atherosclerosis development, it has previously been suggested that VWF has a pathogenic role in atherosclerosis. This hypothesis is supported by results from animal studies (9-11). However, studies in patients with type 3 von Willebrand disease, characterized by a total deficiency of VWF in the circulation, revealed no reduction in atherosclerotic lesions (12-14). The role of VWF in the development of atherosclerosis in humans is therefore still unresolved. In a recent study, we observed a strong association between the extent of atherosclerosis, measured by the calcification volume in the aortic arch and carotid arteries, and VWF levels in ischemic stroke patients (15). Because VWF also plays a pivotal role in platelet aggregation and thrombus formation, these high VWF levels may further increase the risk of coronary events in patients with high risk atherosclerotic lesions.

Intravascular ultrasound (IVUS) can accurately quantify coronary atherosclerosis (16, 17). A previous study in 697 patients with an acute coronary syndrome at inclusion showed that half of the incident recurrent cardiovascular events occurred in patients with non-culprit lesions present at baseline, assessed by IVUS imaging (18). High-risk coronary lesions that are predictive for events include lesions with a plaque burden of at least 70%, a minimal luminal area of 4.0 mm^2 or less or the presence of IVUS virtual histology (VH)-derived thin-cap fibroatheroma lesions (VH-TCFA) (18).

In order to gain further insight into the relationship between VWF levels and cardiovascular outcome, the aim of the present study was to investigate the associations of coronary plaque burden, and the presence of high-risk coronary lesions as assessed by virtual histology intravascular ultrasound (VH-IVUS) with VWF levels, as well as to investigate the association of VWF with 1-year cardiovascular outcome in patients with coronary artery disease (CAD).

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 in detail elsewhere (19). In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for an acute coronary syndrome (ACS) or stable angina pectoris (SAP) have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands.

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 in ClinicalTrials.gov, number NCT01789411.

Von Willebrand Factor measurement

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were transported to the clinical laboratory of the Erasmus MC for further processing and storage at temperature of −80°C within 2 hours after blood collection. VWF antigen (VWF:Ag) levels were determined (N=577) using citrate blood with an in-house ELISA using rabbit anti-human VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging. Reference standard plasma was calibrated against the international standard (Cryocheck Reference, Kordia, Leiden, The Netherlands) and was used as a calibrator. The intra- and inter-assay coefficients of variation were 2.6% and 4.7%.

Intracoronary 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 outcome data. The IVUS 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 (median interslice distance, 0.40 mm). 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 (Figure 1). A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Three types of high-risk lesions were identified: 1. Virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesion, defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen; 2. lesion with large plaque burden, defined as a lesion with a plaque burden of ≥70%; 3. stenotic lesion, defined as a lesion with a minimal luminal area of ≤4.0 mm^2 (Figure 1) (18, 20-22).

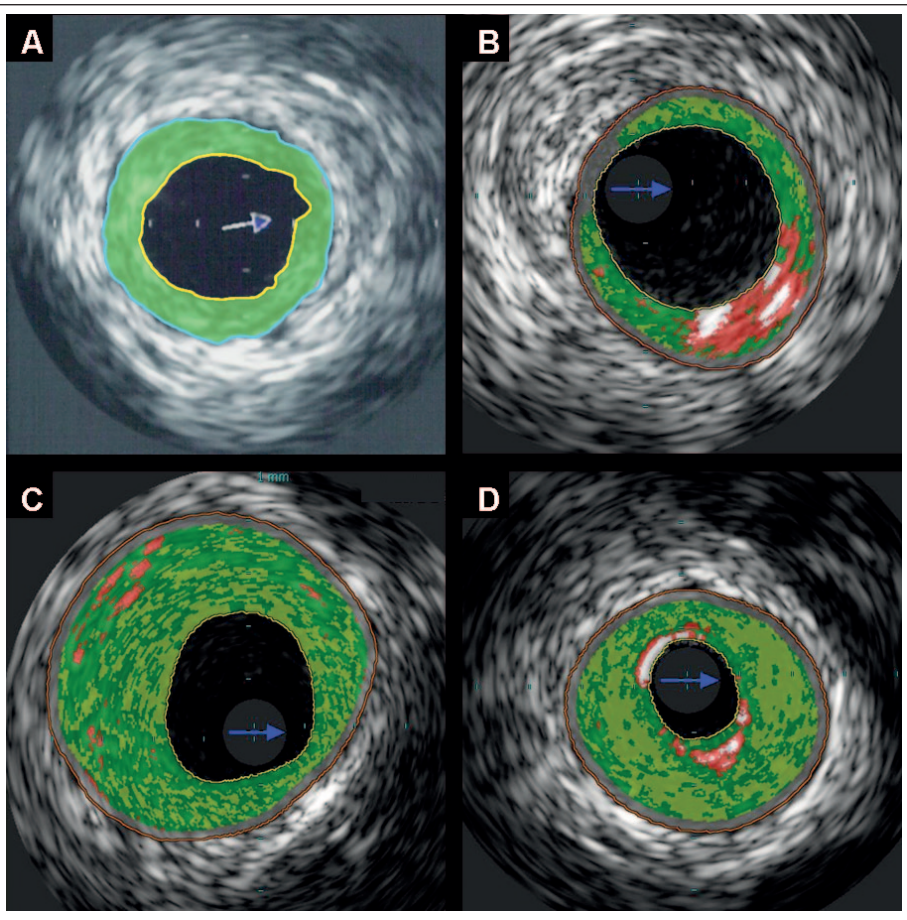

Figure 1. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound virtual histology
A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma lesion, defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. White indicates dence calcium, light green indicates fibrofatty tissue, and dark green indicates fibrous tissue. C: Lesion with plaque burden of ≥70%. D: Lesion with a minimal luminal area of ≤4.0 mm^2.

Clinical 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 contracted for additional information whenever necessary.

The primary endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. 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 (23). Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). The secondary endpoint was defined as the composite of all-cause mortality or ACS. The endpoints were adjudicated by a clinical event committee that had no knowledge of the VWF:Ag levels and IVUS data.

Statistical analysis

The distributions of the continuous variables, including VWF levels and the IVUS parameters, were tested for normality by visual examination of the histogram. Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are presented as median and interquartile range (IQR). VWF levels were not normally distributed and were therefore natural logarithmically (ln) transformed (lnVWF:Ag), where after a normal distribution was acquired. Categorical variables are presented as numbers and percentages. We examined associations of plaque burden and presence of high-risk coronary lesions with VWF:Ag levels. VWF:Ag levels and plaque burden were divided into tertiles. To test for linear association, we used linear regression analyses with continuous ln-transformed VWF:Ag level as dependent variable. In multivariable analyses, the covariates age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking and history of myocardial infarction were considered as established cardiovascular risk factors and as potential confounders, and were therefore entered into the full model.

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. Cox proportional hazards regression analyses were performed to evaluate the associations between VWF:Ag levels and study endpoints. Analyses were adjusted for age, gender and plaque burden. The final results are presented as crude and adjusted hazard ratios (HR) with 95% confidence interval (95% CI).

We a priori expected that there might be heterogeneity in effect estimates between patients with ACS and patients with stable angina pectoris, since VWF:Ag levels are known to be elevated in the acute phase of an ACS (24, 25). Therefore, all statistical analyses were performed separately for patients with ACS and patients with stable angina pectoris at inclusion. 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

In total 577 patients were included, 315 had an ACS and 262 had a SAP. Patients had a mean age of 61.5 years and 75% were men (Table 1). Over half of the patients had single vessel disease. SAP patients had a higher prevalence of cardiovascular risk factors than ACS patients. ACS patients were more likely to smoke. ACS patients had significantly higher VWF:Ag levels than patients with SAP (median 1.73 IU/ml [IQR 1.27-2.31] vs. 1.26 IU/ml [0.93-1.63], p<0.001) (Table 1).

Plaque burden was significantly higher in SAP patients than in ACS patients (39.7 ± 11.0% vs. 36.9 ± 11.8%, p = 0.005). In SAP patients, higher plaque burden was associated with higher VWF:Ag levels (P for trend 0.015) (Figure 2). Also after adjustment for

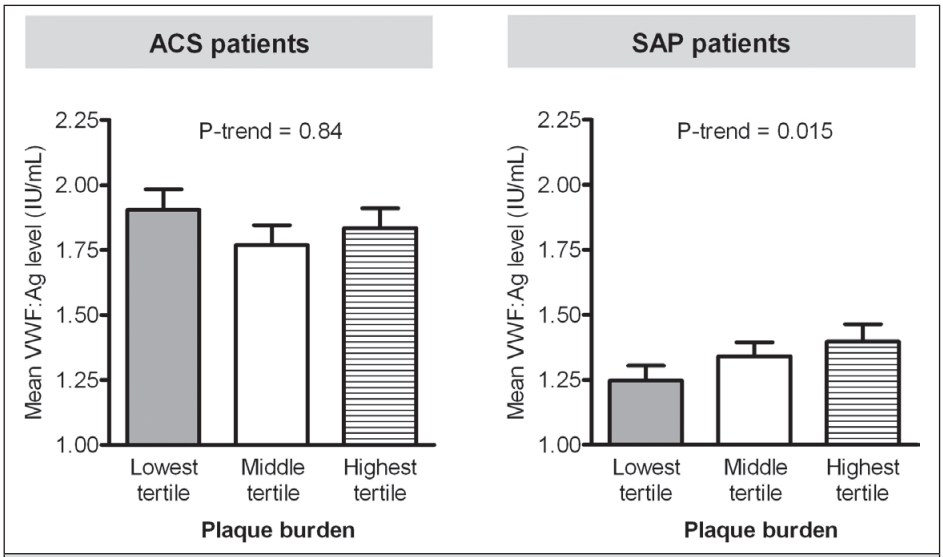

Figure 2. Coronary plaque burden of imaged coronary segment in relation to Von Willebrand Factor levels

Mean ± standard error VWF:Ag levels per tertile coronary plaque burden.

ACS = acute coronary syndrome; SAP = stable angina pectoris; VWF:Ag = von Willebrand Factor antigen.

Table 1. Baseline characteristics

	ACS patients (n=315)	SAP patients (n=262)
Patient characteristics		
Age, years	59.7 ± 11.8	63.6 ± 10.2
Men, n (%)	232 (73.7)	203 (77.5)
Diabetes mellitus, n (%)	40 (12.7)	59 (22.5)
Hypertension, n (%)	138 (43.8)	161 (61.5)
Hypercholesterolemia, n (%)	139 (44.1)	180 (68.7)
Smoking, n (%)	117 (37.1)	50 (19.1)
Positive family history, n (%)	145 (46.0)	155 (59.2)
Previous MI, n (%)	80 (25.4)	104 (39.7)
Previous PCI, n (%)	57 (18.1)	128 (48.9)
Previous CABG, n (%)	7 (2.2)	11 (4.2)
Previous stroke, n (%)	11 (3.5)	15 (5.7)
Peripheral artery disease, n (%)	12 (3.8)	24 (9.2)
History of renal insufficiency, n (%)	13 (4.1)	19 (7.3)
History of heart failure, n (%)	6 (1.9)	13 (5.0)
Von Willebrand Factor, IU/mL	1.73 [1.27-2.31]	1.26 [0.93-1.63]
Procedural characteristics		
Coronary artery disease		
No significant stenosis, n (%)	18 (5.7)	25 (9.5)
1-vessel disease, n (%)	174 (55.2)	133 (50.8)
2-vessel disease, n (%)	88 (27.9)	78 (29.8)
3-vessel disease, n (%)	35 (11.1)	26 (9.9)
PCI performed, n (%)	293 (93.0)	214 (81.7)
IVUS segment characteristics		
Imaged coronary artery		
Left anterior descending, n (%)	120 (38.1)	88 (33.6)
Left circumflex, n (%)	110 (34.9)	84 (32.1)
Right coronary artery, n (%)	85 (27.0)	90 (34.4)
Segment length, mm	44.1 [33.0-54.3]	44.3 [34.3-57.2]

Data are presented as mean ± standard deviation or as median [interquartile range].
ACS = acute coronary syndrome; CABG = coronary artery bypass grafting; MI = myocardial infarction; PCI = percutaneous coronary intervention; SAP = stable angina pectoris.

established cardiovascular risk factors in multivariable analysis, higher plaque burden remained associated with higher VWF:Ag levels (p = 0.027) in patients admitted with SAP. In ACS patients, the coronary plaque burden was not associated with VWF:Ag levels (P for trend 0.84). VWF:Ag levels were not significantly different between patients with and without high risk coronary lesions in both ACS and SAP patients (Figure 3).

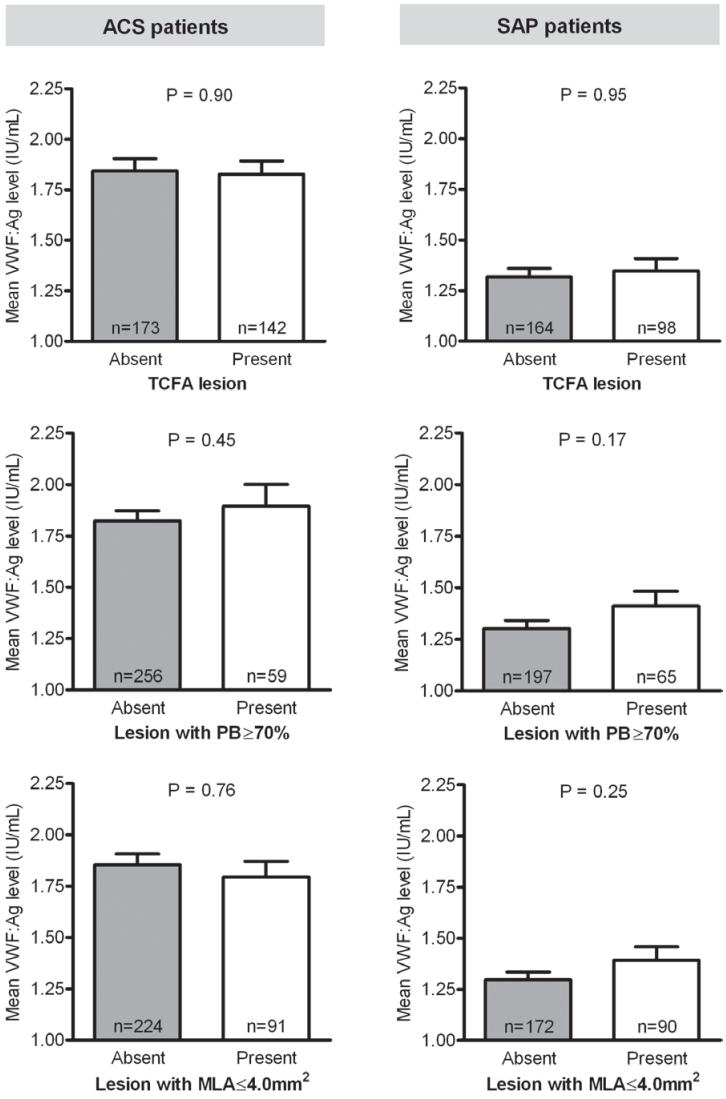

Figure 3. High risk coronary lesions in relation to Von Willebrand Factor levels
Mean ± standard error VWF:Ag levels between high-risk coronary lesions present or absent.
ACS = acute coronary syndrome; MLA = minimal luminal area; PB = plaque burden; SAP = stable angina pectoris; TCFA = thin-cap fibroatheroma; VWF:Ag = von Willebrand Factor antigen.

For 575 (99.7%) patients the vital status at 1-year follow-up could be acquired, and the response rate to the questionnaires that were sent to all living patients was 93.4%. After 1 year of follow-up, 55 patients (9.6%) had experienced a MACE (Table 2). The cumulative Kaplan-Meier incidences of the 1-year MACE was 8.3% for patients with ACS, and 11.1% for patients with SAP. The risk of all-cause death and ACS was significantly associated

Table 2. Number of patients with incident major adverse cardiac events

Number of patients	ACS patients (n=315)	SAP patients (n=262)
Composite of major adverse cardiac events	26	29
Death from any cause	13	4
Definite cardiac or unexplained sudden death	6	2
Acute coronary syndrome	7	7
Myocardial infarction	4	3
Unplanned coronary revascularization	6	18
Composite of death or acute coronary syndrome	20	11

ACS = acute coronary syndrome; SAP = stable angina pectoris.

with higher VWF:Ag levels in both ACS patients (HR 7.45, 95% CI 2.15-25.9, P=0.002) and patients with SAP (HR 7.07 95% CI 1.40-35.6, P=0.018). Additional adjustment for plaque burden did not affect the risk estimate for all-cause death and ACS in ACS patients (HR 4.13 95% CI 1.47-11.6), while the risk in SAP patients was slightly lower (HR 4.05 95% CI 0.88-18.7). Higher VWF:Ag levels were also significantly associated with a higher incidence of MACE in ACS patients (HR 4.14, 95% CI 1.47–11.6, P=0.007), but not in patients with SAP (HR 1.31, 95% CI 0.52-3.29, p=0.57) (Table 3, Figure 4). Additional adjustment for plaque burden did not change the results.

Table 3. Associations between von Willebrand Factor level and cardiovascular outcome

	ACS patients		SAP patients	
	*HR (95%CI)**	*P*	*HR (95%CI)**	*P*
MACE				
Unadjusted	4.28 (1.61-11.4)	0.004	1.39 (0.56-3.42)	0.48
Adjusted for age and gender	4.14 (1.47-11.6)	0.007	1.31 (0.52-3.29)	0.57
Adjusted for age, gender and plaque burden	4.13 (1.47-11.6)	0.007	1.08 (0.43-2.70)	0.87
Composite of death or ACS				
Unadjusted	7.15 (2.21-23.1)	0.001	7.62 (1.58-36.8)	0.011
Adjusted for age and gender	7.45 (2.15-25.9)	0.002	7.07 (1.40-35.6)	0.018
Adjusted for age, gender and plaque burden	7.65 (2.16-27.2)	0.002	4.05 (0.88-18.7)	0.073

* Hazard ratio per SD increase in ln-transformed Von Willebrand Factor level.
ACS = acute coronary syndrome; MACE = major adverse cardiac event; SAP = stable angina pectoris.

DISCUSSION

This is the first study that has investigated the association between invasive measured coronary atherosclerosis by VH-IVUS and VWF:Ag levels. We have shown that patients with an ACS have significantly higher VWF levels than patients with SAP. In patients with

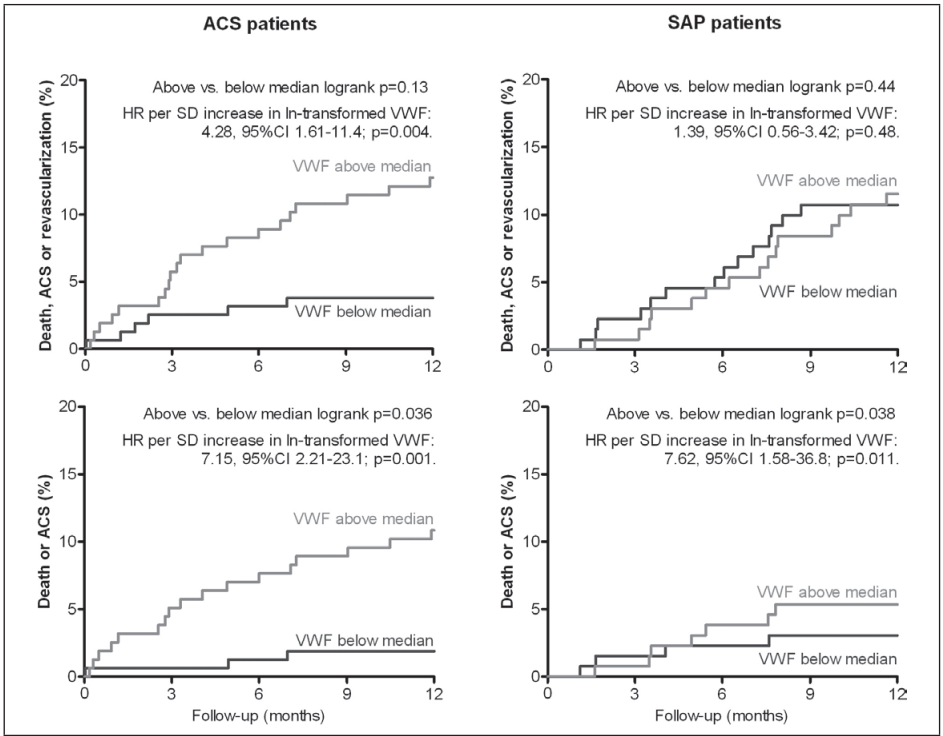

Figure 4. Von Willebrand Factor and cardiovascular outcome
Kaplan-Meier curve for the cumulative event-free survival of MACE or death and hospitalization for ACS per VWF:Ag above and below the median (1.45 IU/ml).
ACS = acute coronary syndrome; SAP = stable angina pectoris.

SAP, coronary plaque burden was positively associated with VWF:Ag levels. In addition, high VWF:Ag levels were associated with death and ACS at 12 months follow up and this was also observed for all MACE in patients with ACS.

The exact pathophysiologic role of VWF in cardiovascular disease has not been elucidated yet. First, it has been hypothesized that VWF may play a causal role in the development of atherosclerosis, thereby increasing the risk of CAD. This was suggested by animal studies with VWF deficient mice, which showed less development of atherosclerosis (9-11). However, human studies, for instance in patients with type 3 von Willebrand disease who have a complete deficiency of VWF, could not confirm these findings (12, 14). However, these patients may incidentally receive VWF concentrates and some use prophylaxis at regular basis and are therefore not completely VWF deficient. It is now suggested that the association between atherosclerosis and VWF is mainly driven by the fact that VWF is a marker of endothelial damage, which is also observed in atherosclerosis (26, 27).

In this study we found that patients with ACS had significantly higher VWF:Ag levels compared with SAP patients, which is in line with a previous study (24). The finding that

plaque burden was associated with VWF:Ag levels in SAP patients confirms our previous findings that VWF is associated with the extent of atherosclerosis. In our previous study in ischemic stroke patients, we observed that a higher calcification volume in the aortic arch and carotid arteries was associated with higher VWF:Ag levels (15). The fact that there was no association between plaque burden and VWF:Ag levels in ACS patients might be explained by the strongly increased VWF:Ag levels in these patients due to an acute phase response, which is well known for VWF (2, 25).

We observed no association between several types of high-risk coronary lesions, including thin-cap fibroatheroma lesions, lesions with plaque burden ≥70% or lesions with a minimal luminal area ≤4.0mm^2 and VWF:Ag levels. High risk lesions are precursors of plaque rupture and may thereby account for the occurrence of coronary thrombi (18, 22, 28). Our results suggest that although VWF is associated with the extent of atherosclerosis, it is not associated with the phenotypic more vulnerable atherosclerotic lesions and might be more involved in stable atherosclerosis. However, a previous mice study showed, by molecular imaging, that activated VWF was found in atherosclerotic disease with high risk features (29). This difference might be explained by the VWF measurement, as only locally activated VWF was measured in the mice study and in our study we measured circulating VWF:Ag plasma levels. In addition, a difference in the pathophysiologic mechanism of destabilising the plaque between mice and human could also influence the results (30-33).

Our data on the association between VWF:Ag levels and MACE in ACS patients strengthens findings of previous studies suggesting that VWF has a predictive role in cardiovascular outcome (34-39). These results were not affected by additional adjustment for plaque burden, suggesting a role for VWF in cardiovascular outcome. In SAP patients, we found an association between high VWF levels and risk of death or ACS. After additional adjustment for plaque burden the association was not significant anymore in SAP patients, which may be explained by the small sample size, resulting in reduced power. These data suggest that the high VWF levels observed in ACS patients, the most severe CAD patients, at inclusion predict MACE at follow-up. However, in the definition of MACE unplanned revascularisation was included which may be considered as a weaker end-point and could therefore have influenced the adverse outcome risk (40). Overall these data supports the role for VWF in the prognosis of patients with a CAD, independent of plaque burden.

There are some limitations of this study. First, blood was sampled in the acute phase at the moment of the coronary angiography. This may explain the higher VWF:Ag levels in ACS patients compared with SAP patients. Therefore, this could have influenced our results. However, we separated the ACS and SAP patients for all analyses. Secondly, a single non-culprit coronary vessel was imaged in this study. This may have led to an underestimation of the association between the presence of high risk lesions in the overall

coronary tree and VWF:Ag levels. However, a previous study have shown that culprit and non-culprit lesions were equally related to MACE (18). In addition, the spatial resolution of IVUS-VH (150 μm) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65 μm) (41). Therefore, IVUS-VH tends to overestimate the number of thin-cap fibroatheroma lesions. Nevertheless, the presence of VH-TCFA lesions has been shown to carry prognostic information (18, 22). Finally, due to the cross-sectional design our data are not able to distinguish whether VWF is causal or a marker of atherosclerosis.

In conclusion, the extent of coronary atherosclerosis is associated with VWF:Ag levels in SAP patients undergoing coronary angiography, but not in ACS patients which might be explained by the acute phase response. High VWF:Ag levels have a predictive role for adverse cardiovascular outcome, and also for MACE in ACS patients, independent of plaque burden.

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CORONARY VULNERABILITY

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HAPTOGLOBIN POLYMORPHISM IN RELATION TO CORONARY PLAQUE CHARACTERISTICS ON RADIOFREQUENCY INTRAVASCULAR ULTRASOUND AND NEAR-INFRARED SPECTROSCOPY IN PATIENTS WITH CORONARY ARTERY DISEASE

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ABSTRACT

Background: Conflicting results exist regarding the association between a common Haptoglobin (Hp) polymorphism and risk of coronary artery disease. We investigated the association of three functionally different anti-oxidant and anti-inflammatory Hp phenotypes (Hp1-1, Hp2-1, Hp2-2) with invasively measured degree and composition of coronary atherosclerosis as determined by intravascular ultrasound (-virtual histology) (IVUS(-VH)) as well as near-infrared spectroscopy (NIRS).

Methods: Non-culprit coronary artery segments of 581 patients with acute coronary syndrome (ACS) or stable angina pectoris were imaged with IVUS(-VH). In 203 patients, the segments were also imaged with NIRS. Pre-procedural blood samples were drawn for Hp phenotyping. Degree (segment plaque volume, segment plaque burden (PB); presence of lesions with PB ≥ 70%) and composition (segment fractions of fibrous, fibrofatty, dense calcium, and necrotic core tissue; presence of IVUS-VH derived thin-cap fibroatheroma lesions) of coronary atherosclerosis were measured.

Results: No differences were present between the three Hp phenotypes with regard to degree and composition of coronary atherosclerosis in the full cohort. However, ACS patients with a Hp2-1 or Hp2-2 phenotype had a higher segment PB percentage (β[95% CI]: 3.88[0.31–7.44], p = 0.033), increased prevalence of lesions with PB ≥ 70% (OR[95% CI]: 3.61[1.06–12.30], p = 0.040), and a tendency towards a higher segment plaque volume (β[95% CI]: 1.29[−0.04–2.62], p = 0.056) in multivariable analyses.

Conclusions: Although in the full cohort no associations could be demonstrated between Hp phenotypes and plaque characteristics, a significant association was present between phenotypes resulting from a genotype containing a Hp2 allele (Hp2-1 or Hp2-2) and a higher degree of atherosclerosis in patients with ACS.

1. INTRODUCTION

Circulating haptoglobin (Hp) is hypothesized to influence atherosclerosis through its anti-oxidant and immunomodulatory properties. Specifically, it prevents hemoglobin-driven oxidative reactions in response to intraplaque hemorrhage, and stimulates a variety of pro- and anti-inflammatory cytokines [1,2]. The Hp gene carries a common polymorphism with two alleles (Hp1 and Hp2), resulting in three functionally-different phenotypes, each characterized by a unique protein structure: Hp1-1 (wildtype genotype; dimer), Hp2-1 (heterozygous variant; linear polymer) and Hp2-2 (homozygous variant; cyclic polymer) [2]. The homozygous variant Hp2-2 produces a dysfunctional protein with the lowest anti-oxidant and anti-inflammatory properties as compared to the proteins encoded by Hp2-1 or Hp1-1 [1,2].

Although the molecular functions of these proteins in the vascular wall have been well investigated and seem to be clear, clinical studies on the association of Hp phenotypes with coronary events have rendered conflicting results [3,4]. In order to further increase understanding of the pathophysiological relation between Hp phenotypes and coronary atherosclerosis, imaging studies using coronary angiography and CT angiography have been performed. However, these have not been able to further elucidate potential mechanisms [5,6]. The imaging techniques used in these studies only enable evaluation of the lumen of the coronary artery. Conversely, radiofrequency intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) enable evaluation and quantification of the arterial wall itself. However, studies on Hp phenotype and invasively-measured coronary atherosclerotic plaque characteristics by IVUS or NIRS are currently lacking.

In the current study, we investigated the relation between Hp phenotype and in-vivo measurements of degree and composition of coronary atherosclerosis by IVUS and NIRS in 581 patients undergoing coronary angiography. Herewith, we aimed to provide additional insights into the pathophysiology concerning Hp and coronary atherosclerosis.

2. METHODS

The rationale and design of the ATHEROREMO-IVUS study and its ATHEROREMO-NIRS substudy have been described in detail elsewhere [7–9]. These studies were approved by the medical ethics committee of the Erasmus MC and performed in accordance with the declaration of Helsinki. All included patients provided written informed consent.

In brief, 581 patients with an indication for coronary angiography due to stable angina pectoris (SAP) or acute coronary syndrome (ACS) underwent IVUS imaging of a nonstenotic segment of at least 40 mm in length in a predefined non-culprit coronary artery with the Volcano™ s5/s5i Imaging System (Volcano Corp., San Diego, USA), using the

Volcano™ Eagle Eye Gold IVUS catheter (20MHz) [7]. The order of preference for selection of the non-culprit coronary artery segment was: 1. Left anterior descending artery; 2. Right coronary artery; and 3. Left circumflex artery. An automatic pullback system was used with a standard pull back speed of 0.5mm per second. Both IVUS grayscale and virtual histology (IVUS-VH) analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, USA) software. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40mm). The degree and composition of each atherosclerotic plaque were assessed. Plaque volume (mm^3) was defined as the total volume of the external elastic membrane occupied by atheroma and normalized for the length of the imaged segment. Plaque burden (%) was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area and is presented as a percentage. Atherosclerotic plaque composition was characterized into fibrous (FI), fibro-fatty (FF), dense calcium (DC) and necrotic core (NC) tissue and expressed as percentages of total plaque volume. Three types of high-risk lesions were identified: 1. Virtual Histology-IVUS derived thin-cap fibroatheroma (VH-TCFA) lesions (presence of N10% confluent necrotic core in direct contact with the lumen); 2. Lesions with plaque burden ≥70%; and 3. Lesions with a minimal luminal area ≤4.0mm^2. Hp phenotypes were successfully determined in 574 of the patients who underwent IVUS(-VH) imaging (Fig. 1). NIRS (InfraReDx, Burlington, Massachusetts, USA) of the same segment was performed in a subset of 191 patients, as well as 12 additional patients that only underwent NIRS, not IVUS [7,9] (Fig. 1). The U.S. Food and Drug

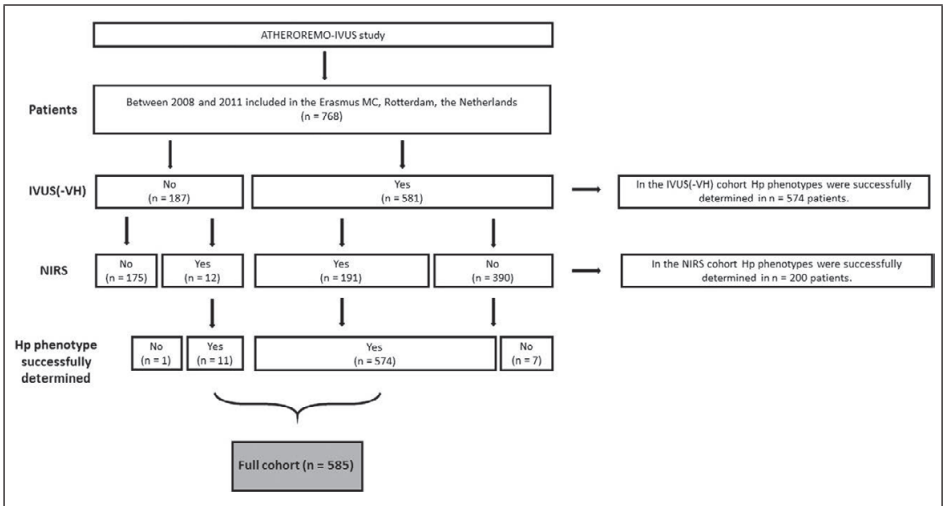

Fig. 1. Flowchart patient inclusion in the ATHEROREMO-IVUS study, ATHEROREMO-NIRS substudy and ATHEROREMO Haptoglobin phenotype substudy.
ACS=acute coronary syndrome; Hp = haptoglobin; IVUS(-VH) = intravascular ultrasound(-virtual histology); NIRS = near-infrared spectroscopy.

Administration-approved NIRS system, as used in this study, consisted of a 3.2-F rapid exchange catheter, a pullback and rotation device. Image acquisition was performed by a motorized catheter pullback at a speed of 0.5 mm per second and 240 rotations per minute. The Lipid Core Burden Index (LCBI) score was measured and represents the amount of lipid core in the imaged segment on a 0-to-1000 scale, as described previously [9]. Both IVUS and NIRS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands), by personnel blinded for baseline patient characteristics and Hp phenotypes. Two-hundred of the 203 patients who underwent NIRS imaging, were successfully phenotyped, resulting in a total of 585 successfully phenotyped and imaged patients for the current investigation (Fig. 1).

EDTA plasma samples were drawn from the arterial sheath prior to coronary angiography and transported to the clinical laboratory of the Erasmus MC for further processing and storage at a temperature of −80°C within 2 h after blood collection. After completion of the cohort, all frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of −80°C) to the Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, where Hp phenotypes were differentiated through western blotting.

The primary endpoint consisted of major adverse cardiovascular events (MACE), and was defined as a composite of all-cause mortality, ACS, and unplanned coronary revascularization. The secondary endpoint consisted of the composite of all-cause mortality and ACS. ACS was defined as the clinical diagnosis of (non-)ST-segment elevation myocardial infarction or unstable angina pectoris [10,11]. Unplanned coronary revascularization was defined as any repeat PCI or coronary artery bypass grafting that was not foreseen at the index procedure. Follow-up data were collected during 1 year. Vital status was obtained from municipal civil registries and questionnaires were sent focusing on the occurrence of MACE to all living patients (response rate of 92.3%). Upon patients' approval, additional information was obtained from hospital discharge letters and treating physicians whenever necessary. Endpoints were adjudicated based on original source data by a clinical events committee.

Variables with a non-normal distribution were transformed by using either the natural logarithm (ln) or square root for further analyses. Univariate analyses were performed by ANOVA or Student's t-test for continuous variables and Chi-squared test for categorical variables, comparing the phenotypes. Multivariate linear and logistic regression analyses were performed with Hp phenotype as the independent variable and with adjustment for the potential confounders age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. Interaction terms were used to test for effect modification by indication for angiography (ACS versus SAP). Subsequently, analyses were stratified on indication. Since ACS subgroup analysis showed (VH-)IVUS values of similar magnitude in the Hp2-1 and Hp2-2 groups, a post-hoc analysis was

performed comparing ACS patients with wildtype Hp1-1 versus a pooled group with the variant phenotypes Hp2-1 and Hp2-2. Furthermore, a subgroup analysis was performed in diabetic patients (n = 99). Finally, the association between Hp phenotype and clinical endpoints after 1 year of follow-up was examined with Cox proportional hazard regression analyses.

All data were analyzed with SPSS software (IBMSPSS Statistics for Windows, Version 23, Armonk, NY, USA). All statistical tests were two-tailed and p-values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Baseline characteristics

Baseline clinical and procedural characteristics of the 3 phenotype groups are presented in Table 1. Prevalence of phenotype Hp1-1, Hp2- 1 and Hp2-2 was 16.1% (n = 94), 45.5% (n = 266), and 38.5% (n = 225), respectively, and this distribution was in Hardy–Weinberg equilibrium (p = 0.67). Mean age ± standard deviation was 61.9 ± 12.6, 62.4 ± 10.7 and 60.0 ± 11.4 years, respectively (p = 0.07). Except for history of myocardial infarction, there were no differences in clinical or procedural characteristics across the various phenotypes. As expected, circulating plasma haptoglobin concentration was lowest for Hp1-1 and highest for Hp2-2 (p=<0.001, Table 1).

3.2. Degree and composition of coronary atherosclerosis

No differences could be demonstrated between the different phenotypes with regard to the degree and composition of atherosclerosis as assessed by IVUS-VH or NIRS (Table 2). The same could be concluded for the subgroup analysis of diabetic patients (n=99, data not shown).

Significant interactions were present between Hp phenotype and indication for angiography (ACS versus SAP) for the association with plaque volume, plaque burden, FI tissue percentage and lesions with PB ≥ 70% (p-values for interaction all <0.05 in uni- and multivariate analysis). In line with this, in ACS patients, phenotypes resulting from a genotype containing a Hp2 allele (Hp2-1 or Hp2-2) were significantly associated with a higher plaque volume (p=0.031) in univariate analysis and tended to be associated with a higher plaque volume in multivariate analysis (β [95% CI]: 1.29 [−0.04–2.62] mm3 increase in (square root transformed) plaque volume for having Hp2-1 or Hp2-2 as compared to Hp1-1, p = 0.056) (Table 3, Fig. 2). Moreover, in ACS patients these phenotypes were independently associated with a larger plaque burden (β [95% CI]: 3.88 [0.31–7.44]% increase in PB for having Hp2-1 or Hp2-2 as compared to Hp1-1, p = 0.033) (Table 3, Fig. 3), as well as an increased prevalence of lesions with PB ≥ 70%

Table 1. Baseline clinical and procedural characteristics of the haptoglobin phenotype groups in the full cohort (n= 585).

Clinical characteristics	Haptoglobin 1-1 (n = 94)	Haptoglobin 1-2 (n = 266)	Haptoglobin 2-2 (n = 225)	P-value[a]
Age, years	61.9 ± 12.6	62.4 ± 10.7	60.0 ± 11.4	0.07
Male gender, n (%)	69 (73.4)	200 (75.2)	173 (76.9)	0.79
Diabetes mellitus, n (%)	11 (11.7)	46 (17.3)	43 (19.1)	0.28
Hypertension, n (%)	48 (51.1)	138 (51.9)	118 (52.7)	0.96
Dyslipidemia, n (%)	45 (47.9)	154 (57.9)	126 (56.3)	0.24
Smoking, n (%)	25 (26.6)	83 (31.2)	63 (28.0)	0.61
Positive family history, n (%)	58 (61.7)	139 (52.3)	110 (49.1)	0.12
Peripheral artery disease, n (%)	4 (4.3)	18 (6.8)	14 (6.2)	0.68
Previous myocardial infarction, n (%)	24 (25.5)	98 (36.8)	64 (28.4)	0.050
Previous PCI, n (%)	26 (27.7)	91 (34.2)	73 (32.4)	0.51
Previous CABG, n (%)	3 (3.2)	9 (3.4)	7 (3.1)	0.99
Previous stroke, n(%)	3 (3.2)	12 (4.5)	10 (4.4)	0.85
History of renal insufficiency, n (%)	4 (4.3)	21 (7.9)	8 (3.6)	0.10
Haptoglobin level, mg/ml	0.79 [0.58–0.99]	1.53 [1.10–2.20]	1.60 [1.10–2.30]	<0.001
Procedural characteristics				
Indication for coronary angiography				
Acute coronary syndrome, n (%)	49 (52.1)	144 (54.1)	127 (56.4)	0.76
Stable angina pectoris, n (%)	45 (47.9)	122 (45.9)	98 (43.6)	0.76
Coronary artery disease				
No significant stenosis, n (%)	4 (4.3)	17 (6.4)	22 (9.8)	0.16
1-vessel disease, n (%)	57 (60.6)	138 (51.9)	117 (52.0)	0.30
2-vessel disease, n (%)	26 (27.7)	82 (30.8)	60 (26.7)	0.58
3-vessel disease, n (%)	7 (7.4)	29 (10.9)	26 (11.6)	0.54
PCI performed	86 (91.5)	233 (87.6)	195 (86.7)	0.48

Continuous variables are presented as mean ± SD or median [interquartile range], depending on their distribution. Categorical variables are presented as n (%).
PCI= percutaneous coronary intervention; CABG= coronary artery bypass graft surgery.
[a] P-values obtained by ANOVA for the continuous variables and Chi-squared test for the categorical variables.

(OR[95% CI]: 3.61 [1.06–12.30], p = 0.040) (Table 3, Fig. 4). With respect to atherosclerotic plaque composition, no associations were present with the various VH-tissue types, LCBI or VH-TCFA lesions in ACS patients (Table 3).

Table 2. NIRS and (VH-)IVUS segment and lesion characteristics of the Haptoglobin phenotype groups in the full cohort (n = 585).

	Haptoglobin 1-1 (n = 94)	Haptoglobin 1-2 (n = 266)	Haptoglobin 2-2 (n = 225)	P-value[c]
Segment plaque characteristics[a]				
Degree of atherosclerosis				
Plaque volume, mm^3	240.7 [118.5–313.4]	235.1 [150.8–332.9]	216.0 [147.6–323.3]	0.94
Plaque burden, %	37.4 ± 12.1	38.3 ± 11.6	38.4 ± 11.1	0.80
Plaque composition				
Fibrous percentage	57.6 ± 12.0	57.4 ± 11.4	58.2 ± 11.7	0.74
Fibro-fatty percentage	9.1 [5.9–12.4]	9.2 [5.9–13.3]	8.4 [5.3–11.9]	0.19
Necrotic core percentage	21.7 ± 8.1	21.0 ± 8.3	21.8 ± 7.7	0.57
Dense calcium percentage	9.5 [5.3–14.4]	9.5 [5.4–15.3]	9.1 [4.9–15.1]	0.92
Lipid Core Burden Index (LCBI)[b]	47.5 [9.0–93.5]	40.5 [16.0–85.8]	40.0 [13.3–80.8]	0.82
Lesion plaque characteristics[a]				
Degree of atherosclerosis				
≥1 Lesion with PB ≥70%, n (%)	18 (20.2)	54 (20.5)	51 (23.2)	0.74
≥1 Lesion with MLA ≤4.0 mm^2, n (%)	29 (32.6)	83 (31.7)	68 (30.9)	0.96
Plaque composition				
≥1 TCFA, n (%)	43 (48.3)	106 (40.3)	91 (41.2)	0.40

Continuous variables are presented as mean ± SD or median [interquartile range], depending on their distribution. Categorical variables are presented as n (%).

PB= plaque burden; MLA = minimal lumen area; and TCFA = thin-cap fibroatheroma.

[a] IVUS(-VH) imaging was performed in 574 patients.

[b] NIRS imaging was performed in a subset of 200 patients.

[c] P-values obtained by ANOVA for the continuous variables and Chi-squared test for the categorical variables.

3.3. Clinical endpoints

With regard to clinical outcome, associations between Hp phenotype and 1-year cardiovascular outcome could not be demonstrated, both in the full cohort and in the ACS and diabetes subgroups. In particular, the Hp phenotypes were not associated with the occurrence of MACE (primary composite endpoint) on multivariate analysis: HR[95% CI] 0.88 [0.52–1.49] for Hp2-1 and 0.97 [0.57–1.67] for Hp2-2 in the full cohort; HR[95% CI] 0.77 [0.38–1.56] for Hp2-1 and 0.70 [0.33– 1.49] for Hp2-2 in the ACS subgroup; HR [95% CI] 0.91 [0.30–2.82] for Hp2-1 and 0.94 [0.30–3.02] for Hp2-2 in the diabetic subgroup.

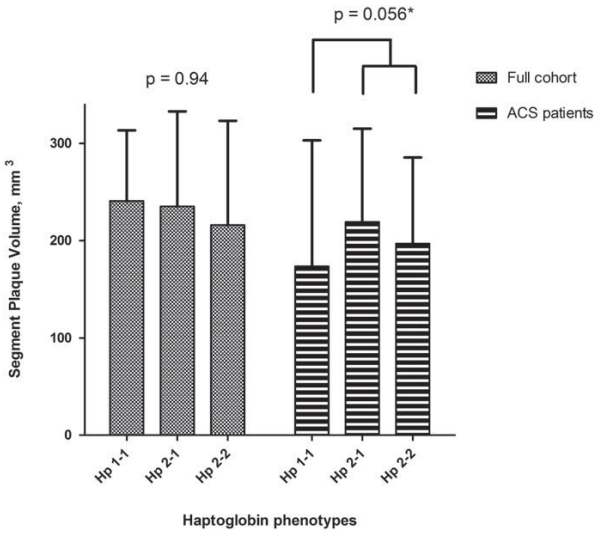

Fig. 2. Segment plaque volume in the haptoglobin phenotypes within the full cohort and within the ACS subgroup.

* P-value for difference in segment plaque volume between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; and Hp = haptoglobin.

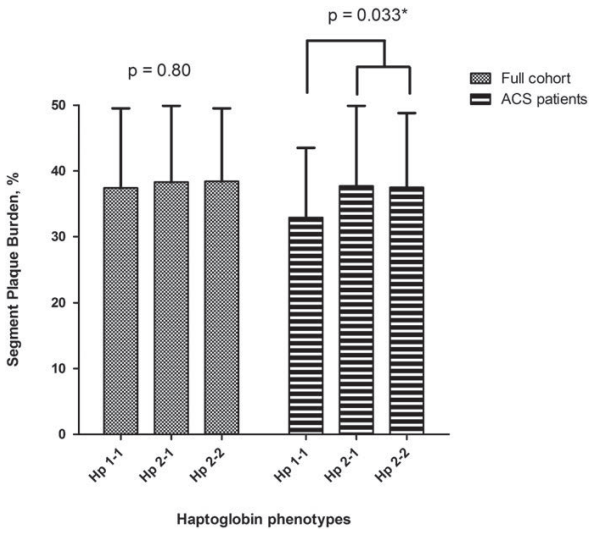

Fig. 3. Segment plaque burden in the haptoglobin phenotypes within the full cohort and ACS subgroup.

*P-value for difference in segment plaque burden between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; Hp= haptoglobin.

Table 3. NIRS and (VH)-IVUS segment and lesion characteristics of the haptoglobin phenotype groups in patients with ACS (n = 320).

	Haptoglobin 1-1 (n = 49)	Haptoglobin 2-1 or 2-2 (n = 271)	P-value[c]	β [95% CI]	P-value[d]
Haptoglobin level, mg/ml	0.84 [0.63–1.10]	1.60 [1.10–2.50]	<0.001	0.65 [0.45–0.85]	<0.001
Segment plaque characteristics[a]					
Degree of atherosclerosis					
Plaque volume, mm^3	173.5 [107.3–303.1]	215.5 [141.2–304.2]	0.031	1.29 [–0.04–2.62]	0.056
Plaque burden, %	32.9 ± 10.6	37.6 ± 11.8	0.014	3.88 [0.31–7.44]	0.033
Plaque composition					
Fibrous percentage	61.4 ± 11.2	58.5 ± 12.0	0.13	–2.99 [–6.80–0.83]	0.12
Fibro-fatty percentage	8.6 [4.6–12.0]	8.6 [5.5–12.0]	0.35	0.18 [–0.14–0.51]	0.26
Necrotic core percentage	21.2 ± 8.3	21.8 ± 8.6	0.68	0.43 [–2.37–3.22]	0.76
Dense calcium percentage	6.7 [4.9–11.3]	8.3 [4.9–13.8]	0.28	0.21 [–0.17–0.58]	0.28
Lipid Core Burden Index (LCBI)[b]	48.0 [6.0–91.0]	44.5 [16.0–88.0]	0.53	0.03 [–0.71–0.77]	0.93
Lesion plaque characteristics[a]			P-value[c]	OR [95% CI]	P-value[d]
Degree of atherosclerosis					
≥1 Lesion with PB ≥ 70%, n (%)	3 (6.7)	56 (21.0)	0.023	3.61 [1.06–12.30]	0.040
≥1 Lesion with MLA ≤4.0 mm^2, n (%)	11 (24.4)	80 (30.1)	0.44	1.30 [0.61–2.7	0.51
Plaque composition					
≥1 TCFA, n (%)	22 (48.9)	119 (44.6)	0.59	0.78 [0.41–1.51]	0.46

Continuous variables are presented as mean ± SD or median [interquartile range], depending on the distribution. Categorical variables are presented as n (%).

Beta (β) indicates the increase or decrease (minus sign) in each (transformed) imaging segment parameter for the Haptoglobin 2-1 or 2-2 ACS patients as compared to Haptoglobin 1-1 ACS patients.

Odds ratio (OR) increase in each lesion parameter for the Haptoglobin 2-1 or 2-2 ACS patients as compared to Haptoglobin 1-1 ACS patients.

[a] IVUS-VH imaging was performed in 313 ACS patients.

[b] NIRS imaging was performed in a subset of 93 ACS patients.

[c] P-values (univariate) obtained by the independent Student's two-sample t-test for the continuous variables and Chi-squared test for the categorical variables.

[d] P-values (multivariate) obtained by linear regression analyses for continuous variables and logistic regression analyses for categorical variableswith Haptoglobin 1-1 as the reference category. Models adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction.

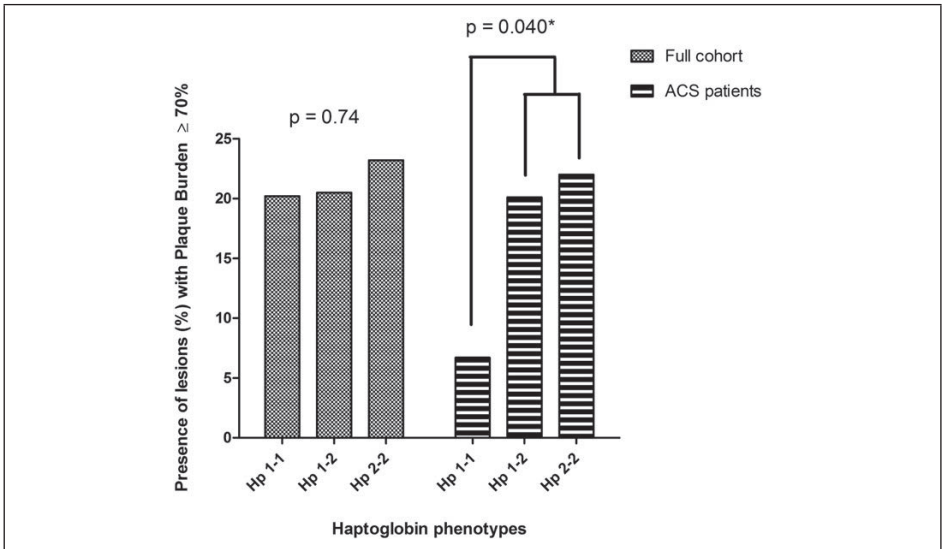

Fig. 4. Presence of large lesions in the haptoglobin phenotypes within the full cohort and ACS subgroup.

*P-value for difference in the presence of large lesions between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; Hp= haptoglobin.

4. DISCUSSION

To our knowledge, this is the first study that investigated the relation between Hp phenotypes and coronary plaque characteristics as assessed with IVUS-VH and NIRS in patients with CAD. Although no associations could be demonstrated between Hp phenotypes and coronary plaque characteristics in the full cohort, in ACS patients phenotypes Hp2-1 and Hp2-2 were significantly associated with a higher degree of coronary atherosclerosis as expressed by higher segment plaque burden and higher prevalence of lesions with PB ≥ 70%, as compared to Hp1-1.

Existing imaging studies on Hp phenotype and atherosclerosis as assessed with either coronary angiography or CT angiography are limited in number and were mainly performed in specifically defined study populations, such as patients with diabetes mellitus [5,6,12,13]. Overall, these studies did not find any associations between Hp phenotype and coronary atherosclerosis [5,6,12,13]. The interaction between Hp phenotype and acute versus stable clinical presentation of CAD has not been investigated earlier. A potential, biologically plausible explanation for this interaction could be that patients who ultimately experience ACS represent a subgroup that exhibits an increased pro-inflammatory [14] and oxidative state [15–17] as compared to SAP patients. In these patients, elevated oxidative stress may not only be present systemically [15,16] but also

locally at the level of the atherosclerotic plaque, as part of the pathogenesis and evolution towards an ACS. The latter results, among others, from intraplaque hemorrhage, which occurs more often in ACS than in SAP patients, and gives rise to a local release of hemoglobin (iron) into the atherosclerotic plaque [18]. Such a state leads, among several other reactions, to local generation of reactive oxygen species and consequently lipid peroxidation [15], which eventually may contribute to accelerated atherosclerotic plaque growth [15,19] in these patients. This process may be further enhanced in Hp2 phenotypes (Hp2-1 and Hp2-2) due to their reduced anti-oxidant and anti-inflammatory properties as compared to Hp1-1 [1,2]. A previous study in mice supports this hypothesis by demonstrating increased iron, lipid peroxidation and macrophage accumulation in Hp2-2 atherosclerotic plaques as compared with Hp1-1 plaques [20]. This was confirmed in humans by autopsy studies that have demonstrated more advanced atherosclerotic plaques in Hp2-2 compared to Hp1-1 individuals [21]. In contrast to Hp1-1 proteins, the Hp2-1 and Hp 2-2 proteins have low affinity for both hemoglobin and the macrophage CD163 scavenger receptor in order to clear hemoglobin (iron) from the atherosclerotic plaque and prevent its harmful intraplaque oxidative reactions [2,22]. Altogether, these studies indicate that oxidative stress might strongly be implicated in the atherosclerotic process with a critical role for Hp proteins in its further development.

In a previous study within the same cohort, we could not demonstrate an association between plasma Hp concentration and (VH-)IVUS plaque characteristics or clinical events [8]. Although the biological function of Hp in the vascular wall might not directly depend on its plasma concentrations, but rather on its protein structure, there is a direct correlation of Hp phenotype with Hp plasma concentrations. Specifically, Hp concentration is higher in Hp2-2 than in Hp1-1 individuals, because of the weaker binding of Hp2-2 proteins to hemoglobin and the macrophage CD163 receptor [22]. Thus, since Hp concentrations may at least in part be phenotype-dependent, these negative results seem consistent with our current findings.

Epidemiologic studies investigating the association between Hp phenotype and incidence of CAD in the general population are limited in number and have yielded contradicting results. While De Baquer et al. found that Hp1-1 individuals were at higher risk of CAD mortality as compared to the other Hp phenotypes [3], the Framingham Heart Offspring Study (n=3273) could not demonstrate any relationship between Hp phenotype and CAD prevalence in the overall study population [4].

The majority of clinical studies concerning Hp phenotypes has focused on diabetic individuals, since strong evidence exists that Hp phenotype and diabetic state significantly interact with regard to prevalence of CAD. It has been demonstrated that Hp2-2 individuals with diabetes have a higher risk of adverse cardiovascular outcomes as compared to the other phenotypes [4,23–27], which is thought to be caused by the decreased anti-oxidant capabilities of the Hp2-2 protein in conjunction with an exception-

ally high level of oxidative stress in diabetes [28,29]. However, some other studies could not confirm these findings [30], or even rendered contradictory results [4]. We also could not demonstrate any associations with in-vivo coronary plaque characteristics or 1-year clinical outcome in diabetic patients. Our findings are in agreement with a study in type 2 diabetic patients, that could not demonstrate an association between Hp genotype and coronary artery calcification (CAC) as a reflection of total coronary atherosclerotic burden [5]. On the other hand, a larger case-control study on type 1 diabetic patients found that the Hp2-2 genotype was a predictor of CAC progression. The limited number of diabetic participants (n = 99) in our cohort may have contributed to the lack of such an association in our study.

Our study has several limitations that warrant acknowledgement. Firstly, our findings in the ACS subgroup should be considered as hypothesis-generating, because the comparison of Hp2-2 and Hp2-1 on the one hand with Hp1-1 on the other hand in the ACS patients was a post-hoc analysis. Nevertheless, the interaction terms between Hp phenotypes and indication for catheterization were highly significant in multivariate analyses. Secondly, IVUS(-VH) imaging took place of a non-culprit coronary artery segment only. However, this approach was developed under the hypothesis that such a non-culprit target segment adequately reflects coronary wall pathophysiology of the larger coronary tree, and this hypothesis has been confirmed by several studies [31,32]. Finally, this study was not primarily designed to investigate the association between Hp phenotypes and atherosclerosis and clinical outcome in diabetic patients. A small number of diabetic patients in this study may have contributed to the lack of significant associations between Hp phenotypes and degree and composition of atherosclerosis in this subgroup.

In conclusion, in patients undergoing coronary angiography, no associations were present between Hp phenotypes and invasively measured coronary atherosclerotic plaque characteristics by IVUS and NIRS. However, patients with Hp2-1 or Hp2-2 presenting with ACS had a significantly higher degree of coronary atherosclerosis as compared to Hp1-1. Thus, genetic differences in the endogenous antioxidant status, as reflected by the haptoglobin phenotype, may be of considerable importance in patients suffering from CAD. Our hypothesis-generating findings should be confirmed by other, large studies in order to identify patient groups that might benefit from risk stratification by Hp phenotyping in the future.

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Conflict of interest

None.

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CORONARY VULNERABILITY

IV

INTERVENTION STUDIES

CORONARY VULNERABILITY

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DARAPLADIB EFFECT ON CIRCULATING HIGH SENSITIVE TROPONIN IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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ABSTRACT

Objectives: We compared the incidence of late increase in hs-cTnI between ACS and non-ACS patients treated with standard of care with or without darapladib.

Methods: A total of 323 (161 ACS and 162 non-ACS patients) were included. High sensitivity troponin I was measured at baseline and at 4, 13, 26 and 52 weeks.

Results: ACS patients had statistically higher hs-cTnI values during longer term follow-up at which these patients were no longer in the acute setting of myocardial ischemia, but were regarded to have stable CAD (mean hsTnI value in ACS patients: 1.180 versus 0.886 ng/L in non-ACS patients, $p = 0.02$). Multivariate logistic regression revealed three predictors of any 2-fold increase in hs-cTnI levels compared to the previous visit when interactions were not considered. Treatment with darapladib (adjusted OR 0.53; 95% CI: 0.30-0.92) and initial presentation with ACS (adjusted OR 0.42; 95% CI: 0.23-0.77) were associated with less frequent occurrence of a 2-fold increase in hs-cTnI levels. In contrast, diabetes was associated with a higher incidence of 2-fold increases in hs-cTnI levels (adjusted OR 2.20; 95% CI: 1.04-4.64). Logistic regression to predict any 2-fold increase in hs-cTnI by ACS status showed that in the ACS group, treatment with darapladib decreased the risk of elevation of hs-cTnI (OR 0.219; 95% CI: 0.087, 0.553, $p = 0.0013$).

Conclusion: In patients with ACS, treatment with darapladib is associated with less increase in cardiac troponin I compared to standard of care alone. This beneficial effect may be associated with darapladib's capability of reducing necrotic core in coronary plaques.

1. INTRODUCTION

Myocardial damage after either temporal or permanent suppression of the coronary blood flow has been reported to be prognostically relevant for patients. [1] Early detection of myocardial damage is highly encouraged to better assess risk of a new clinical event. Several markers of myocardial damage have been described (i.e creatinine kinase and cardiac troponin – cTn) as means of diagnosis of myocardial infarction [2]. Elevations of serum cardiac troponin levels above the detection limit have been associated with increased mortality and recurrent ischemic events in patients with acute coronary syndrome (ACS) and also in subjects without clinical evidence of cardiovascular disease [3-5]. As a consequence, the possibility that circulating troponin levels below the conventional detection limits might lead to further risk stratification for adverse cardiovascular outcome, led to the development of so-called high-sensitivity troponin (hs-cTn) assays. A post-hoc analysis of the PEACE trial demonstrated that, in patients with stable coronary artery disease (CAD), cardiac troponin T concentrations as measured with a highly sensitive assay were significantly associated with increased incidence of cardiovascular mortality and heart failure after adjustment for other independent prognostic indicators [6].

In addition, the serial assessment of c-Tn could also be a prognostically relevant marker of late events and its late suppression could become a therapeutical target.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a circulating enzyme bound predominantly to apoB-containing lipoproteins, and highly expressed in the necrotic core of atherosclerotic lesions [7,8]. Lp-PLA_2 rapidly degrades oxidatively modified phospholipids in LDL-c leading to formation of proinflammatory and cytotoxic products [9–11]. Because enhanced cell death and impaired clearance of apoptotic bodies are thought to be key mechanisms for necrotic core expansion [12], Lp-PLA_2 inhibition may favorably affect rupture-prone lesions.

Darapladib (GlaxoSmithKline, Philadelphia PA) which is a Lp- PLA_2 inhibitor has been studied in the Integrated Biomarkers and Imaging Study-2 trial (NCT00268996) [13]. One of the key findings was that Lp-PLA_2 inhibition with darapladib interferes with necrotic core expansion.

The primary objective of this exploratory post-hoc analysis is to compare hs troponin I levels between darapladib and placebo subjects in the IBIS 2 study, and to describe the differences in patterns of troponin levels between patients presenting with ACS and those presenting with non-ACS as measured longitudinally with a high sensitivity immunoassay during one-year follow-up.

2. METHODS

2.1. Study design

The Integrated Biomarkers and Imaging Study-2 trial has been published elsewhere [13]. Briefly, it was an international, multicenter, randomized, double blind, placebo-controlled study in patients with confirmed CHD. Institutional review boards at each center approved the protocol, and patients provided written informed consent.

2.2. Patient population

Patients 18 years of age or older undergoing cardiac catheterization for acute coronary syndrome (ACS) or non-ACS were eligible.

In the protocol, ACS is defined as: patients with enzymatic evidence of myocardial necrosis [chest pain or chest pain equivalent lasting greater than or equal to 20 min within past 72 h with elevated pre-catheterization levels of troponin I or T (i.e. >99th percentile of reference control group)]. Non-ACS is defined as: patients with coronary heart disease other than troponin-positive acute coronary syndrome.

Randomization allocated patients into darapladib vs. placebo groups and was stratified according to ACS status and center. Key exclusion criteria were planned surgical revascularization, stroke in the past 6 months, chronic hepatic disorder or abnormal ALT, bilirubin (ALT >2.5 or bilirubin >1.5 upper limit of normal), serum creatinine >2.0 mg/dL, blood pressure >160/100 mm Hg, poorly controlled diabetes mellitus (HbA_{1c} >10%), severe heart failure or left ventricular ejection fraction <30%, and current life-threatening condition. Patients were ineligible if angiography demonstrated left main coronary stenosis >50% or coronary anatomy was inappropriate for IVUS.

2.3. IVUS imaging

The ECG-gated IVUS-RF acquisition was performed using EagleEye catheter (20 MHz) at pullback speed of 0.5 mm/s as described. The quantitative IVUS analysis was performed by the Core Imaging Laboratory (Cardialysis, Rotterdam, The Netherlands) using customized software (pcVH 2.1, Volcano Therapeutics). After selection of the region of interest in the nonculprit vessel, vessel and lumen area data were obtained for every cross-section throughout the region of interest by semiautomatic planimetry of the leading edges of the luminal and external elastic membrane borders. Necrotic core was identified with autoregressive classification system that showed sensitivity and specificity of 92% and 97% for detection of necrotic core, respectively. The intra- and interobserver variability of necrotic core measurements: the mean absolute difference for necrotic core area was 0.01 mm^2 (SD 0.06) for the intraobserver and 0.02 mm^2 (SD 0.08) for the interobserver variability, respectively.

2.4. Biomarkers

Plasma samples were drawn at baseline (prior to the cardiac catheterization), weeks 4, 13, 26, 52 and at the follow-up visit. Cardiac troponin I was measured using the ultrasensitive Singulex Erenna System (Singulex Inc., Berkeley, CA, USA) which is an ultrasensitive flow-based immunoassay that uses single-molecule counting [14]. It has been standardized to National Institute of Standards and Technology Material and validated with a lower limit of detection of 0.0002 ng/mL (0.20 ng/L). The inter-assay coefficient of variation (CV) is 10% at 0.91 ng/L, and the 99th percentile in a healthy control population is 9 ng/L [15].

2.5. Statistical analysis

Baseline characteristics are reported as mean values (+/– standard deviation) for continuous variables, whereas discrete variables are presented in terms of frequencies and percentages.

Summary statistics were calculated for the natural log of the area under the curve (AUC) high sensitive troponin I, weighted by day, excluding baseline and week 4 (thus from week 13 to week 52). Treatment group comparisons were based on the general linear model with terms for treatment group and ACS status. Pearson correlations between the change from baseline to week 52 in necrotic core volume and the weighted area under the curve of hs troponin (excluding the baseline and week 4 visits) were calculated.

The proportions of subjects with 2-,3- and 4- fold increases in hs-cTnI from the previous visit were examined by treatment group and ACS status. These proportions were examined excluding the baseline value and the week 4 hs-cTnI values because ACS subjects would be expected to have high hs-cTnI at entry into the study. Thus, solely the late increase/suppression (>13 weeks) was explored.

Logistic regression modeling was performed to predict any 2- fold increase in hs-cTnI from the previous visit. The following terms were included in the model: treatment group, ACS status, treatment group by ACS status interaction, age, smoking status, presence of a stent at baseline, previous MI, hypertension, HDL <1.03, LDL <1.81 mmol/L, and diabetes. In this model, only treatment group, ACS status, and the treatment group by ACS status interaction were significant at the 5% level. Due to the significant interaction between treatment group and ACS status, odds ratios for treatment group are presented within ACS status.

All statistical tests were two-sided with a type I error level of 0.05. Analyses were performed with SAS version 9.1.

3. RESULTS

A total of 323 patients constituted the Intent-to-Treat population (patients who took at least 1 dose), 161 patients had an acute coronary syndrome (ACS) and 162 had a non-ACS. At baseline, 252 patients had at least one stenting procedure in a non-study vessel. Table 1 contains the baseline characteristics of patients in the ITT population.

Table 1. Baseline characteristics

Clinical characteristics	Placebo (*n* = 151)	Darapladib (*n* = 172)
Age (y)	57.3 ± 10.9	59.4 ± 9.8
Males (*n*, %)	126 (83)	140 (81)
Body-mass index (kg/m^2)	27.8 ± 3.8	27.5 ± 4.0
Diabetes mellitus (*n*, %)	22 (15)	22 (13)
Hypertension (*n*, %)	89 (59)	115 (67)
Low HDL cholesterol (<40 mg/dL) (n, %)	40 (26)	45 (26)
Hypercholesterolemia (*n*, %)	95 (63)	108 (63)
Current smoker (*n*, %)	57 (38)	64 (37)
Prior medical history (*n*, %)		
Prior myocardial infarction	49 (32)	51 (29)
Prior coronary revascularization	47 (31)	50 (29)
Peripheral artery disease	7 (5)	17 (10)
Prior stroke	3 (2)	4 (2)
Index hospitalization (*n*, %)		
PCI during index hospitalization	122 (81)	130 (76)
ACS	74 (49)	87 (51)
STEMI	35 (23)	40 (23)
Non-STEMI	39 (26)	47 (27)
Cardiovascular medications at randomization (*n*, %)		
Aspirin	138 (91)	149 (87)
Clopidogrel or ticlopidine	122 (81)	136 (79)
Any antiplatelet medication	150 (>99)	170 (99)
ACE inhibitors or ARBs	88 (58)	101 (59)
Beta-blockers	119 (79)	138 (80)
Statins	134 (89)	157 (91)
Laboratory values		
Cholesterol (mg/dL)		
Total	187.3 ± 47.6	182.3 ± 43.2
LDL	108.2 ± 41.4	103.6 ± 37.4
HDL	46.8 ± 11.2	48.0 ± 12.4

Table 1. *(continued)*

Clinical characteristics	Placebo (n = 151)	Darapladib (n = 172)
Triglycerides (mg/dL)		
Median	141	136
IQR	97-202	96-193
hsC-reactive protein (mg/L)		
Geometric mean	2.4	2.4
95% CI	1.9, 3.1	1.9, 3.0
Lp-PLA_2 activity ($\mu mol/min^{-1}/L^{-1}$)[a]		
Geometric mean	159	160
95% CI	152, 167	153, 167
Blood pressure		
Systolic-mm Hg	125.7 ± 16.9	128.0 ± 16.1
Diastolic-mm Hg	75.2 ± 10.1	75.6 ± 9.9
Study vessel[b]-no. (%)		
LAD	44 (36)	56 (39)
LCX	32 (26)	37 (26)
RCA	45 (37)	51 (35)
Diameter stenosis[c] (%)	28.0 (10.5)	26.6 (10.3)
Mean lumen diameter[c] (mm)	2.9 ± 0.5	3.0 ± 0.6

Values are presented as mean ± SD unless otherwise specified; to convert to mmol/L multiply values of cholesterol by 0.02586 and triglycerides by 0.0113; PCI, percutaneous coronary intervention; ACS, acute coronary syndromes.

[a] Plasma Lp-PLA2 activity was measured by a colorimetric method with an intraassay precision of 1.7% and interassay precision of 4.8%.

[b] Imaging evaluable population: placebo 121 patients; darapladib 146 patients.

[c] Quantitative coronary angiography: placebo 121 patients; darapladib 144 patients.

Higher high sensitivity troponin AUCs were observed in patients presenting with an ACS as compared to those without (mean hsTnI values: 3.7 ± 1.8 versus 1.1 ± 0.1.2 ng/L, $p < 0.001$), when measurements at all timepoints were taken into account. (Fig. 1) ACS patients also had statistically higher hs-TnI values during longer term follow-up, at which these patients were no longer in the acute setting of myocardial ischemia (>4 weeks), but were regarded to have stable CAD (mean hsTnI value in ACS patients: 1.180 versus 0.886 ng/L in non-ACS patients, $p < 0.02$).

3.1. High-sensitivity troponin I levels over time

When hsTnI measurements at all timepoints were considered, 18% of ACS subjects vs. 33% of non-ACS subjects had a 2-fold increase in hsTnI levels compared to the previous sample collection. As mentioned earlier, hsTnI levels are known to be elevated at

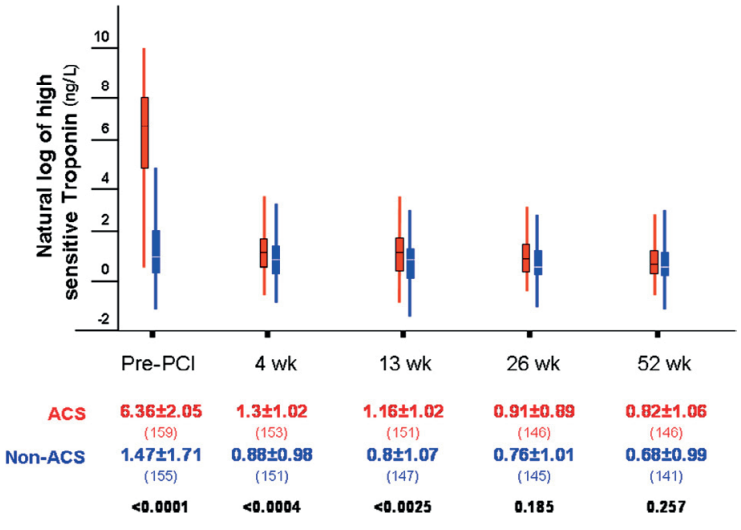

Fig. 1. High sensitivity troponin I levels by visit and acute coronary syndrome (ACS) status.

baseline in the ACS patients, followed by a period of normalization (i.e. decrease instead of increase). A second model was therefore used to assess whether the lower incidence of 2-fold increases in hsTnI levels would persist in the ACS patients when only changes between visits from week 13 onwards were evaluated. This also demonstrated that 2-fold increases in hsTnI levels during follow-up occurred less frequently in ACS patients (15% of ACS subjects vs. 26% of non-ACS subjects) (Online Supplement Table 1).

Multivariate logistic regression revealed three predictors of any 2-fold increase in hsTnI levels compared to the previous visit. After adjustment for clinically relevant variables, treatment with darapladib (adjusted OR 0.53; 95% CI: 0.30-0.92) and initial presentation with ACS (adjusted OR 0.42; 95% CI: 0.23-0.77) were associated with less frequent occurrence of a 2-fold increase in hsTnI levels. In contrast, diabetes was associated with a higher incidence of 2-fold increases in hsTnI levels (adjusted OR 2.20; 95% CI: 1.04-4.64). No other clinical baseline characteristic was significantly associated with hsTnI increases, either in univariate or multivariate analyses. (Table 2) However, due to the presence of a significant treatment by ACS status interaction, a logistic regression model to predict any 2-fold increase in troponin by ACS status was performed. In the ACS group, treatment with darapladib reduced the risk of hs-TnI elevation (OR 0.219; 95% CI: 0.087, 0.553, $p = 0.0013$) (Table 3).

No significant correlations were found between change in necrotic core volume and the weighted area under the curve of hscTnI in the overall study population or in subgroups of ACS-status or treatment allocation (Online Supplement Table 2).

Table 2. Details of logistic regression modeling to predict any 2-fold increase in troponin from the previous visit full model (no interaction terms).

	Estimate	*p*-value	OR (95% CI)
Treatment group (darapladib vs placebo)	−0.32	0.024	0.526 (0.301, 0.919)
ACS status (ACS vs non-ACS)	−0.43	0.005	0.420 (0.230, 0.765)
Age	0.005	0.745	1.005 (0.976, 1.034)
Smoker (yes vs no)	−0.03	0.857	0.944 (0.502, 1.773)
Stent at baseline (yes vs no)	−0.25	0.138	0.609 (0.316, 1.173)
Previous MI (yes vs no)	−0.12	0.423	0.780 (0.425, 1.433)
Hypertension (yes vs no)	−0.14	0.366	0.763 (0.424, 1.373)
HDL (<1.03 vs >=1.03)	0.13	0.447	1.305 (0.657, 2.595)
LDL (<1.81 vs >=1.81)	−0.21	0.157	0.663 (0.375, 1.172)
Diabetes (yes vs no)	0.40	0.038	2.200 (1.044, 4.636)

Table 3. Details of logistic regression modeling to predict any 2-fold increase in troponin from the previous visit model by ACS status.

	Estimate	*p*-value	OR (95% CI)
ACS subgroup ($n = 159$)			
Treatment group (darapladib vs placebo)	−0.7583	0.0013	0.219 (0.087, 0.553)
Non-ACS subgroup ($n = 159$)			
Treatment group (darapladib vs placebo)	−0.1228	0.4683	0.782 (0.403, 1.519)

Additionally, examination of the incidence of clinical events in those patients who had a 2-fold increase of hs-cTnI from the previous visit ($n = 80$), showed that 27.5% of these patients had at least one MACE (cardiovascular death, MI, stroke, coronary revascularization) event. (Table 4). The timing of the determination of hsTnI and MACE event is reported in the Online Supplement Table 3. A 2-fold increase in hs-cTnI was not related to the date of the MACE events in the majority of events.

4. DISCUSSION

In this exploratory post-hoc analysis of the IBIS 2 trial, there was a marked suppression of the late elevation (>13 weeks) of high sensitivity troponin I in patients treated with darapladib. This was more apparent in patients that had ACS at the time of randomization to darapladib, when compared with non-ACS patients.

In a substudy from the FRISC-II trial(5), persistent minute elevation (levels >0.01 μg/L) of cTnI, predicted mortality during long-term follow-up. In our study, an association with mortality cannot be done due to the nature of the IBIS study (i.e. imaging vs. outcome trial). Nevertheless, it is expected that treatment with darapladib may have an effect

Table 4. **Summary of subjects with a MACE event and a two-fold increase in troponin in IBIS-2.**	
Subjects with MACE	58/323 (18%)
Death	0
Myocardial infarction	11
Stroke	2
Coronary revascularization	57
Subjects with 2 MACE	13
Subjects with a two-fold increase in troponin from the previous visit	80/323 (25%)
Subjects with a two-fold increase in troponin from the previous visit:	
And a MACE event[a]	22/80 (27.5%)
And no MACE event	58/80 (72.5%)

Note: MACE includes death, myocardial infarction, stroke, and coronary revascularization.
[a] Includes subjects with both a MACE and a two-fold troponin increase at any time. Troponin increase may have been before or after MACE.

on clinical outcomes not only by preventing late elevations of troponin (this report), but also for halting progression of necrotic core assessed by IVUS-virtual histology [13]. One can hypothesize that the late elevation of cardiac TnI is caused by several factors: 1. increased demand ischemia due to volume and pressure overload that can occur in patients with high prevalence of co-morbidities (congestive heart failure, left ventricular hypertrophy, diabetes mellitus, and chronic kidney disease) [16,17]. In the context of a randomized control trial such as IBIS 2, the distribution of these diseases is expected to be equal in both treatment arms; 2. Apoptosis of cardiomyocytes could be another explanation for measurable troponin levels in the long term after index procedure. This phenomenon is specific for patients who had an ACS in whom the apoptotic processes persist for months after an AMI. [18]. In humans, circulating Lp-PLA2 is bound predominantly to LDL. Lp-PLA2 acts on oxidized phospholipids within modified LDL to generate lysophosphatidylcholine and oxidized fatty acids. Both products have proinflammatory effects that contribute to the initiation and progression of atheroma, in large part, through the recruitment and activation of monocyte-macrophages. The products of Lp-PLA2 activity can also induce apoptosis among macrophages. Although the exact mechanism has not been yet completely elucidated, monocytes activated by transient hypoxia protect cardiomyocytes during hypoxia and re-oxygenation through expression of CD11b receptors [19]. Thus, this process can be affected by darapladib by preventing the formation of lysophosphatidylcholine and oxidized fatty acids, thereby avoiding monocyte apoptosis and therefore protecting myocytes; 3. Late TnI increase is marked in non-ACS patients treated with and without darapladib, and in ACS patients treated with standard of care, but not in those ACS patients treated with darapladib. Whether ACS patients receiving darapladib represent a subset of patients with enhanced

reduction in major adverse cardiovascular events is currently being investigated in the SOLID-TIMI 52 trial [20]. As mentioned above, a possible explanation is that ACS patients exhibit a more significant inflammatory process post-ACS with activation of monocytes and macrophages, as compared with non-ACS patients. In such environment darapladib is hypothesized to play a major role, reducing the apoptosis of these cells with eventual protection of cardiomyocytes. 4. Asymptomatic coronary plaque ruptures with subsequent microembolization of the resulting thrombus may also represent a cause of late increase in hs-cTnI. It has been reported that patients with ACS have additional asymptomatic ruptured plaques beyond the culprit lesions, showing the multifocal nature of the disease [21]. The size of the necrotic core is one important determinant for the rupture of those plaques [22]. In the IBIS 2, the progression of the expansion of necrotic core was halted by darapladib [13]. In this subset of patients (i.e. ACS), darapladib also decreases the incidence of a two-fold increase in high sensitive troponin; 5. Another simpler reason for the late increase in cTn is the occurrence of thrombotic events in this population. In this report, the 2-fold increase in hsTnI occurred in 25% (80/323) of the total population, and of these patients only 27.5% (22/80) had at least a MACE event. The raise in high sensitive troponin was most of the time unrelated to the time of the event (Online Supplement Table 3).

4.1. Study limitations

This exploratory post-hoc subanalysis has several limitations: 1. blood samples were not processed at short-term which might have caused increase variability in the assessment of the high sensitivity troponin I as it has been described [23,24]; 2. this report includes the total population included in IBIS 2 study but the sample size is too small to investigate a potential relationship between elevations in high sensitivity troponin and clinical events and; 3. likewise, the observations regarding the effect of darapladib on levels of high sensitive troponin are hypothesis-generating and require further exploration.

5. CONCLUSIONS

In patients with acute coronary syndrome, addition of darapladib to standard of care therapy is associated with a lower incidence of a two-fold increase in cardiac troponin I over time when compared to standard of care alone. This beneficial effect may be associated with darapladib's capability of reducing necrotic core in coronary plaques, and thus warrants further study.

APPENDIX

Core Laboratories: imaging (Cardialysis, Rotterdam, The Netherlands).
Participating Centers (number of patients enrolled): Austria: Hanusch Krankenhaus, Georg Gaul [6]. Belgium: Centre Hospitalier Universitaire Sart-Tilman, Victor Legrand [10]; ZNA Campus Middelheim, Stefan Verheye (25); Cardiovascular Center, Aalst, William Wijns [14]. Czech Republic: V_seobecná Fakultní Nemocnice, Michael Aschermann [23]. Denmark: Skejby University Hospital, Hans Erik Bøtker [18]. Germany: West German Heart Center, Raimund Erbel [7]; Kerckhoff Klinik, Christian Hamm [7]; Universitätsklinikum Heidelberg, Stefan Hardt, Helmut Kücherer (1); Universitätsklinikum München, Volker Klauss [14], Universitätsklinikum Ulm, Wolfgang Koenig [9]; Segeberger Kliniken, Gert Richardt [3]. The Netherlands: Medisch Spectrum Twente, Clemens von Birgelen [14]; Medisch Centrum Leeuwarden, Adrianus Johannes van Boven [12]; Catharina Hospital and Catherine R&D, Herman Rolf Michels [14], Erasmus Medical Center, Patrick Serruys [20]; Medisch Centrum Rijnmond Zuid, Pieter Smits [11]. Norway: Haukeland Sykehus, Oyvind Bleie [20]. Poland: Upper Silesian Heart Center, Pawel Buszman (40); Szpital Uniwersytecki, Dariusz Dudek [19]. Spain: Hospital Marques de Valdecilla, Thierry Colman [9]; Hospital Clinico San Carlos, Carlos Macaya [9]. Switzerland: Kantonsspital Luzern, Paul Erne (25).

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2012.06.064.

Disclosures

Jennifer Shannon and Rich Davies are employees of GlaxoSmithKline. The rest of the authors declare no conflicts of interest relevant to the content of this paper.

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SUPPLEMENTARY MATERIAL

Table 1. Summary of subjects with an increase in high sensitive troponin from the previous visit at weeks 26, 52 by acute coronary syndrome (ACS) Status

	Placebo		Darapladib		Total	
ACS	**71**		**83**		**154**	
2-Fold	17	24%	6	7%	23	15%
3-Fold	11	15%	3	4%	14	9%
4-Fold	6	8%	3	4%	9	6%
Non-ACS	**73**		**80**		**153**	
2-Fold	20	27%	20	25%	40	26%
3-Fold	7	10%	15	19%	22	14%
4-Fold	6	8%	8	10%	14	9%

Table 2. Correlation between the Change from Baseline to Week 52 in Necrotic Core Volume and Weighted Area Under the Curve of hs Troponin (Excluding the Baseline and Week 4 Visits)

	n	Pearson Correlation
Overall	239	0.109 (p=0.09)
Placebo Treatment Group	110	0.115 (p=0.23)
Darapladib Treatment Group	129	0.133 (p=0.13)
ACS	118	0.172 (p=0.06)
Placebo Treatment Group	56	0.182 (p=0.18)
Darapladib Treatment Group	62	0.249 (p=0.05)
Non-ACS	121	–0.048 (p=0.60)
Placebo Treatment Group	54	–0.071 (p=0.60)
Darapladib Treatment Group	67	–0.131 (p=0.29)

ACS, acute coronary syndrome; hs, high sensitive

Table 3. Summary of Subjects with a MACE event and a two-fold increase in Troponin in IBIS-2

Subjects with MACE and two-fold increase in troponin	22
Time from first MACE to two-fold increase in troponin:	
Increase prior to MACE	
>=100 days prior	4
>50 to <100 days prior	2
... 1 day prior	1
Two-fold increase followed first MACE or same day as MACE	
Same day as MACE	3
<=10 days after MACE	2
>10-<30 days after MACE	7
… >100 days after MACE	3

Note: MACE includes death, myocardial infarction, stroke, and coronary revascularization

CORONARY VULNERABILITY

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22

INTEGRATED BIOMARKER AND IMAGING STUDY 3 (IBIS-3) TO ASSESS THE ABILITY OF ROSUVASTATIN TO DECREASE NECROTIC CORE IN CORONARY ARTERIES

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ABSTRACT

Aims: Statins are highly effective in reducing major adverse clinical events, but the direct effects on coronary plaque composition remain debatable. Our aim was to mechanistically evaluate the treatment effect of high-intensity statin therapy on compositional coronary plaque changes.

Methods and results: The third Integrated Biomarker and Imaging Study (IBIS-3) was a prospective, investigator-initiated, single-center study. Serial radiofrequency intravascular ultrasound (RF-IVUS) measurements of a predefined non-stenotic segment in a non-culprit coronary artery were performed to evaluate the effect of rosuvastatin (intended dose: 40 mg daily) on necrotic core (NC) volume in patients with stable angina or acute coronary syndrome. Changes in lipid core burden index (LCBI) were evaluated through serial near-infrared spectroscopy (NIRS) imaging in a subset.

Serial RF-IVUS (and NIRS) data of a median segment of 41 (interquartile range: 32 to 49) mm were complete in 164 (103) patients. Follow-up measurements were performed at 6 and 12 months in 30 (26) and 134 (77) patients, respectively. Mean levels of low-density lipoprotein cholesterol decreased by 30%, from 2.49 mmol/l to 1.73 mmol/l at the end of follow-up. High-dose rosuvastatin therapy resulted in a non-significant (P=0.074) change of −1.4 mm^3 (95% confidence interval [CI]: −3.0 to 0.1) in NC volume during follow-up. The change in NC *percentage* of total plaque volume was −1.4% (95% CI:−2.4 to −0.4; P=0.006). A neutral effect was also observed on LCBI. Indications of significant regression of NC volume and LCBI in the highest baseline quartiles were observed, which should be cautiously regarded as hypothesis generating.

Conclusion: High-intensity rosuvastatin therapy during 1 year resulted in a neutral effect on NC and LCBI within non-stenotic, non-culprit coronary segments with a relatively low atheroma burden.

Keywords: Atherosclerosis, Statin, Radiofrequency Intravascular Ultrasonography, Near-Infrared Spectroscopy

INTRODUCTION

The presence of coronary plaque phenotypes with large necrotic core (NC) volumes is associated with a high incidence of major adverse cardiac events.(1-3) In the second Integrated Biomarker and Imaging Study (IBIS-2), the lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitor darapladip – added to statins – halted coronary NC volume progression.(4) We now report IBIS-3, evaluating high-dose rosuvastatin to reduce coronary NC volume, assessed by radiofrequency intravascular ultrasound (RF-IVUS), and intracoronary cholesterol accumulation, assessed by near-infrared spectroscopy (NIRS).(5)

METHODS

The IBIS-3 study details have been published elsewhere.(5) Briefly, patients undergoing coronary angiography (CAG) or percutaneous coronary intervention (PCI) were treated with high-dose (40 mg daily) rosuvastatin for 12 months. Near completion of the study, the protocol was amended to enable a treatment duration of 6 months.

IBIS-3 was approved by the Medical Ethics Committee of the Erasmus MC. Written informed consent was obtained from all participants.

Subsequent to the index CAG/PCI, RF-IVUS was performed in a non-culprit coronary segment with the Volcano Corporation Eagle-Eye catheter, and NIRS with the InfraReDx system, at a pullback speed of 0.5 mm/sec. Initially, the NIRS system was non-CE marked and several patients refused to provide consent for its use. Intracoronary imaging was repeated at the end of the scheduled rosuvastatin treatment period. RF-IVUS and NIRS images were analyzed offline by an independent core laboratory (Cardialysis, Rotterdam, The Netherlands).

The primary endpoint was the change in NC volume. Secondary endpoints included the change in NC percentage, and the change in NIRS-derived lipid core burden index (LCBI) for the entire region of interest (ROI), and the 10- and 4 mm segments with the highest LCBI, the $LCBI_{max10mm}$ and $LCBI_{max4mm}$, respectively.

We aimed to enroll 300 patients. Assuming an attrition rate of 15%, the sample size was determined at 350 patients.(5) The actual attrition rate appeared to be approximately 30% (Figure 1). We therefore decided to terminate patient enrollment in June 2013.

The study design paper specified that treatment effects be tested with paired Student's t-tests.(5) However, because study endpoints had non-normal distributions, we decided to perform non-parametric statistics instead. Furthermore, we decided to square our data analysis methods with IBIS-4 study, including the use of linear mixed models and regression.(6) We report changes in serum cholesterol levels and study end-

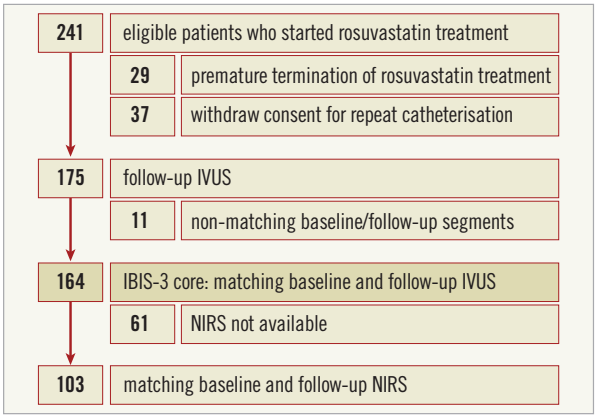

Figure 1. Study flowchart describing inclusion, attrition and the final IBIS-3 core of 164 patients with matching baseline and follow-up RF-IVUS. IVUS: radiofrequency intravascular ultrasound.

points as follow-up minus baseline values, and negative values indicate a decrease over time. All statistical tests were two-sided, and a P-value <0.05 was considered statistically significant.

RESULTS

Serial RF-IVUS was available in 164 patients, including 103 with serial NIRS (Figure 1). Table 1 shows baseline characteristics. Rosuvastatin was taken during a median of 372 (interquartile range: 357 to 395) days, with 90.9% of the patients being titrated to the maximum dose. At the time of the recatheterization, 92% of patients were on rosuvastatin 20-40 mg (online Table 1).

Mean LDL-C decreased by 30%, from 2.49 to 1.73 mmol/l, and HDL-C increased by 11%, from 1.11 to 1.23 mmol/l (Table 2; online Figure 1).

NC volume changed with −1.4 mm^3 (95% confidence interval [CI]: −3.0 to 0.1; Table 2; Figure 2). NC percentage of total plaque volume changed with −1.4% (95% CI:−2.4 to −0.4). The latter finding should be interpreted in conjunction with a modest, but significant rise in percent atheroma volume (PAV). The change in serum LDL-C levels was not associated with the change in coronary plaque characteristics (online Table 2; online Figure 2). Regression of NC volume was observed in patients within the highest baseline quartile (online Table 3; Figure 2).

Within the 103 patients with repeat NIRS, changes in LCBI were non-significant (Table 2; Figure 3). LCBI regression might be pronounced in the highest baseline quartile (online Table 3; Figure 3). There was no correlation between LDL-C change and LCBI

Table 1. Baseline characteristics.

	IBIS-3 core: patients with completed treatment phase and matching baseline and follow-up RF-IVUS (N=164)	Patients without matching follow-up RF-IVUS* (N=77)	*p*-value
Age, years	60.4 (55.3, 65.9)	57.5 (51.6, 66.0)	0.22
Male	84.1	79.2	0.35
Diabetes mellitus	20.7	20.8	0.99
Hypertension	64.2	54.6	0.15
Hypercholesterolaemia	63.6	61.8	0.80
LDL-c, mmol/l	2.41 (1.89, 3.00)	2.69 (1.99, 3.50)	0.030
HDL-c, mmol/l	1.09 (0.91, 1.30)	1.01 (0.91, 1.30)	0.43
Total cholesterol, mmol/l	3.99 (3.29, 4.61)	4.48 (3.60, 5.21)	0.024
Statin use¶	95.1	92.2	0.37
Current smoker	28.0	37.7	0.13
Positive family history	54.6	64.5	0.15
Previous MI	29.9	33.8	0.54
Previous PCI	36.0	40.3	0.52
Previous CABG	0.6	0	1.0
Previous stroke	9.1	13.0	0.36
Peripheral artery disease	4.3	13.0	0.014
History of renal insufficiency	3.7	6.5	0.33
History of heart failure	1.2	1.3	0.96
Indication for coronary angiography			0.009
STEMI	14.7	31.6	
NSTE ACS	26.8	22.4	
Stable angina	58.5	46.1	
Extent of coronary artery disease			0.97
No significant stenosis	3.7	3.9	
1-vessel disease	51.2	49.4	
2-vessel disease	39.0	39.0	
3-vessel disease	6.1	7.8	
PCI performed	89.0	87.0	0.65

Continuous data are presented as median (25th, 75th percentile) values. Categorical data are presented as percentages. *39 patients with premature termination of rosuvastatin treatment, 27 with withdrawal of consent for repeat catheterisation. An additional seven patients did complete the treatment phase and underwent repeat catheterisation, but had non-matching baseline/follow-up segments. ¶ 12 (63%) of the 19 statin naïve patients had no history of vascular disease, as compared to 51% of statin users. CABG: coronary artery bypass grafting; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; MI: myocardial infarction; NSTE ACS: non-ST-elevation acute coronary syndromes; PCI: percutaneous coronary intervention; RF-IVUS: radiofrequency intravascular ultrasound; STEMI: ST-elevation myocardial infarction

Table 2. Baseline and follow-up serum cholesterol and intracoronary imaging endpoints.

	Baseline		Follow-up		Change	
	mean (SD)	median (IQR)	mean (SD)	median (IQR)	mean (95% CI)	*p*-value*
LDL-c, mmol/l	2.49 (0.85)	2.36 (1.92, 2.99)	1.73 (0.71)	1.60 (1.26, 2.01)	–0.76 (–0.91, –0.61)	<0.001
HDL-c mmol/l	1.11 (0.31)	1.07 (0.90, 1.29)	1.23 (0.37)	1.18 (0.97, 1.46)	0.12 (0.08, 0.16)	<0.001
Total cholesterol, mmol/l	4.11 (0.93)	4.0 (3.3, 4.6)	3.34 (0.87)	3.3 (2.7, 3.8)	–0.77 (–0.93, –0.61)	<0.001
External elastic membrane volume, mm^3	579.6 (278.0)	520.8 (376.6, 724.9)	577.0 (273.4)	518.3 (378.1, 715.6)	–2.7 (–9.4, 4.0)	0.42
Lumen volume, mm^3	335.4 (149.7)	314.8 (227.6, 409.1)	329.2 (145.8)	309.4 (225.5, 403.4)	–6.6 (–12.0, –1.2)	0.015
Atheroma volume, mm^3	243.9 (151.3)	204.0 (142.7, 304.8)	247.8 (148.6)	210.9 (145.4, 301.8)	3.9 (–0.2, 8.0)	0.064
Percent atheroma volume, %	40.7 (10.2)	41.5 (32.9, 48.8)	41.6 (9.7)	41.5 (33.8, 49.8)	1.0 (0.4, 1.5)	0.001
NC volume, mm^3	29.1 (31.9)	17.8 (7.3, 38.0)	27.7 (31.2)	19.2 (6.2, 35.1)	–1.4 (–3.0, 0.1)	0.074
DC volume, mm^3	13.0 (15.9)	7.9 (2.3, 17.4)	13.4 (16.9)	8.2 (2.2, 17.2)	0.4 (–0.4, 1.2)	0.31
FI volume, mm^3	71.1 (63.9)	51.3 (31.1, 93.4)	70.8 (61.8)	52.8 (30.6, 94.6)	–0.3 (–2.7, 2.2)	0.83
FF volume, mm^3	13.7 (14.6)	9.0 (3.9, 18.8)	15.7 (15.3)	10.9 (5.4, 22.1)	2.0 (0.6, 3.4)	0.005
NC percentage, %	20.2 (8.2)	20.0 (15.2, 25.0)	18.9 (7.3)	19.5 (14.6, 24.0)	–1.4 (–2.4, –0.4)	0.006
DC percentage, %	9.0 (5.6)	8.4 (4.6, 12.7)	9.1 (5.9)	8.4 (4.4, 13.1)	0.0 (–0.6, 0.7)	0.85
FI percentage, %	60.0 (11.0)	60.5 (52.6, 66.8)	58.7 (11.0)	60.7 (50.6, 66.2)	–1.2 (–2.6, 0.2)	0.076
FF percentage, %	10.7 (5.2)	9.9 (7.5, 13.7)	13.2 (9.9)	11.8 (8.5, 15.6)	2.6 (1.1, 4.1)	0.001
LCBI, full region of interest	44.9 (51.1)	33.0 (6.0, 67.0)	46.1 (43.2)	35.0 (8.0, 72.0)	1.2 (–8.5, 11.0)	0.80
LCBImax10mm	127.8 (121.7)	107.0 (25.0, 197.0)	130.5 (114.0)	109.0 (30.0, 194.0)	2.7 (–16.9, 22.2)	0.79
LCBImax4mm	201.9 (163.8)	182.5 (60.0, 319.0)	206.8 (154.5)	192.0 (72.0, 323.0)	4.9 (–21.7, 31.4)	0.72

*based on linear mixed models (patient as random intercept) to test if change is different from 0. CI: confidence interval; DC: dense calcium tissue; FF: fibro-fatty tissue; FI: fibrous tissue; HDL-c: high-density lipoprotein cholesterol; IQR: interquartile range; LCBI: lipid core burden index; LDL-c: low-density lipoprotein cholesterol; NC: necrotic core tissue; SD: standard deviation

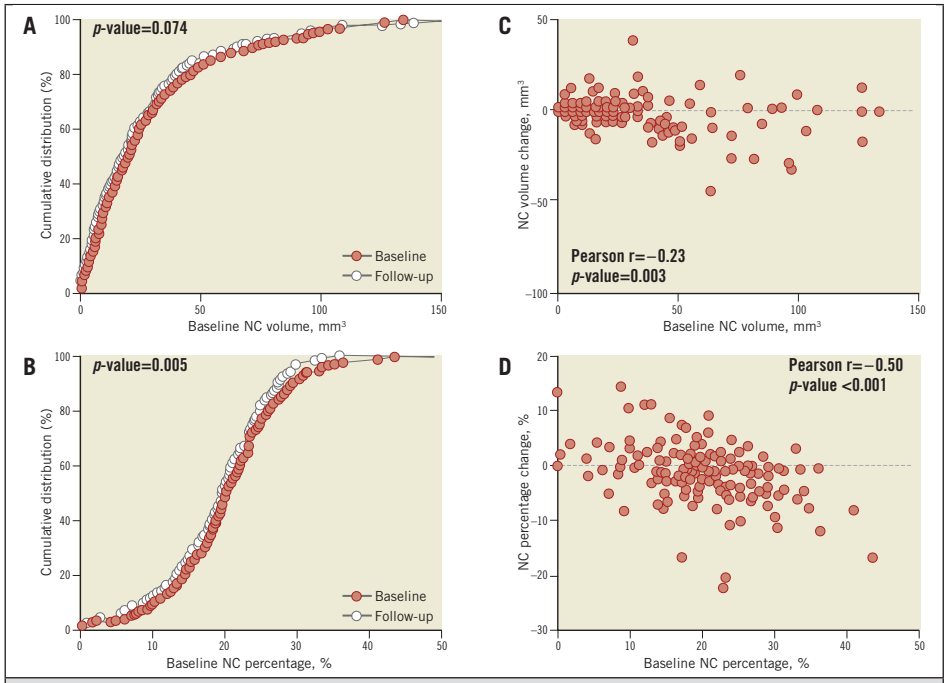

Figure 2. Necrotic core volume and percentage at baseline and follow-up.
High-intensity rosuvastatin therapy led to a neutral effect on NC volume (A) and a significant decrease in NC percentage (B). The highest reductions were observed in those patients with a relatively high necrotic core burden at baseline. Panel C depicts the change of NC under high-intensity rosuvstatine therapy against the baseline NC volume. Panel D illustrates the same for NC percentage.

change (online Table 2; online Figure 2). LCBI change was similar in statin-naïve patients and previous statin users.

All effects were similar in patients with repeat imaging at 6 and 12 months (online Tables 4-6).

DISCUSSION

High-intensity rosuvastatin therapy resulted in a neutral effect on NC and LCBI within non-stenotic coronary segments with a relatively low baseline atheroma burden. IBIS-2 showed a stabilization of NC volume by darapladib, with 91% of patients on statin therapy.(4) IBIS-3 suggests that NC stabilization might be possible with a potent statin alone.

Our findings concur with a meta-analysis of 17 studies involving 2171 patients on at least six different statins, which showed that longer-duration and higher-intensity statin therapy may result in plaque volume regression, but not in a significant NC reduction.

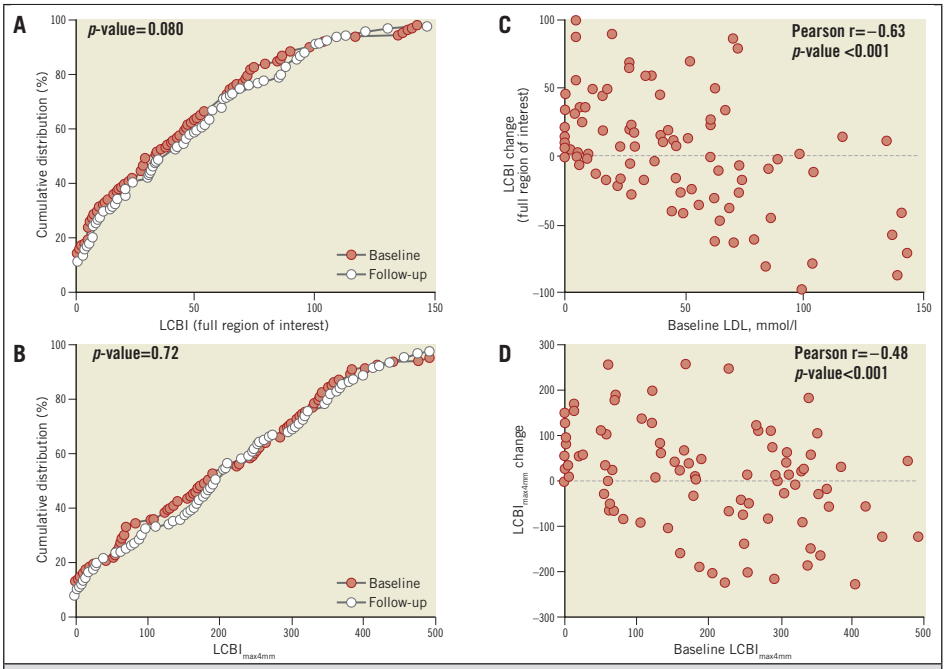

Figure 3. LCBI at baseline and follow-up.
The effect of high-intensity rosuvastatin therapy on LCBI of the full region of interest (A) and the $LCBI_{max4mm}$ (B). Panels C and D indicate that the degree of LCBI regression might be dependent on the baseline LCBI. LCBI: lipid core burden index; $LCBI_{max4mm}$: the 4 mm segment with the highest LCBI.

(7) Lack of change in NC burden after high-intensity statin therapy was also observed in SATURN (8) and in IBIS-4, which studied STEMI patients.(6)

The YELLOW trial demonstrated a significant LCBI reduction in 44 patients after 6-8 weeks of high-intensity rosuvastatin therapy.(9) In the comparator group of 43 patients, who were kept on their 'regular' statin, LCBI remained unchanged. However, YELLOW evaluated the effect of rosuvastatin on untreated obstructive coronary lesions with a fractional flow reserve < 0.8. In contrast, we studied non-flow-limiting coronary segments with a low median LCBI of 33 (versus 95-132 in YELLOW). As a consequence, high-intensity statin therapy in IBIS-3 only had a limited substrate with respect to regression of LCBI. Still, our observation of a significant LCBI reduction in patients with high baseline values might be relevant, since they are at increased risk of adverse cardiac events.(10)

The fact that changes in NC and LCBI were not correlated to changes in serum LDL-C levels may support the abundance of data on the pleiotropic effects of statins that are not directly related to serum lipid levels.(11) We only studied the effect of rosuvastatin on plaque composition in relation to its effect on LDL-C. However, recent studies suggest that LDL-C will not be atherogenic until it becomes oxidized in the arterial wall.(11)

IBIS-3 was an uncontrolled, observational study, similar to IBIS-4 and ASTEROID.(6,12) A disadvantage of such approach is that true treatment effects cannot be distinguished from 'regression to the mean'. In our study, the most pronounced regression of plaque components occurred within the highest baseline quartiles, which might be an expected and logical consequence of a real treatment effect. On the other hand, the simultaneous increase in most plaque parameters that was observed in the lowest baseline quartiles is suggestive for at least a component of regression to the mean.

IBIS-3 was designed to be embedded in our routine clinical practice, which we consider important for external validity. Consequently, however, the IBIS-3 patients were somewhat older and had more comorbidities than observed in similar studies with repeat imaging,(12,13) which may explain their higher than expected drop-out rate. We enrolled 164 of 300 planned patients with repeat IVUS. The observed 1.4 mm^3 NC reduction was smaller than anticipated, but the standard deviation was also smaller (10.0 versus 13.9 mm^3). Consequently, the power of IBIS-3 was still high enough (90%) to declare the anticipated 2.5 mm^3 NC reduction statistically significant, but too small (50%) with regard to the observed effect.

Conclusion

The IBIS-3 study, a prospective, mechanistic, single-arm, open-label study designed to evaluate the treatment effect of high-intensity rosuvastatin therapy, demonstrated a neutral effect on NC volume in a non-culprit coronary artery segment without significant luminal narrowing. Indications of regression of NC percentage and NC volume and LCBI in the highest baseline quartiles should only be cautiously regarded as hypothesis generating.

FUNDING

IBIS-3 was sponsored by AstraZeneca (Wilmington, Delaware, USA), by InfraredX (Burlington, Massachusetts, USA) and Volcano Corporation (San Diego, California, USA). The study was initiated by the authors, and was designed, conducted, interpreted, and reported independently of these sponsors.

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SUPPLEMENTAL DATA

Online Table 1. Rosuvastatin treatment.			
Days to start treatment			23 (18, 31)
Starting dose, mg		5	0.6
		10	84.2
		20	14.6
		40	0.6
Maximum dose, mg		20	9.1
		40	90.9
Days to maximum dose			52 (45, 62)
Total duration of rosuvastatin use, days			372 (357, 395)
Dose at day of repeat catheterisation, mg	rosuvastatin discontinuation		4.9
		5	0.6
		10	2.4
		20	24.4
		40	67.7

Continuous data are presented as median (25th, 75th percentile) values. Categorical data are presented as percentages.

Online Table 2. Study endpoints by quartiles of LDL cholesterol change.					
Study endpoint	Δ LDL-c<−1.33	−1.33≤Δ LDL-c <−0.68	−0.68≤Δ LDL-c <−0.24	−0.24≤Δ LDL-c	*p*-value for trend*
Atheroma volume, mm^3	7.51 (−1.82, 16.8)	1.50 (−6.58, 9.58)	−1.77 (−9.92, 6.39)	10.5 (1.98, 19.0)	0.15
Percent atheroma volume, %	1.40 (0.20, 2.60)	0.51 (−0.59, 1.61)	1.07 (−0.04, 2.17)	1.23 (0.04, 2.43)	0.38
NC volume, mm^3	−0.04 (−2.21, 2.14)	−1.58 (−4.02, 0.87)	−1.77 (−4.11, 0.58)	−1.50 (−6.59, 3.59)	0.71
NC percentage, %	0.53 (−1.45, 2.50)	−0.94 (−2.02, 0.15)	−1.89 (−3.34, −0.44)	−2.83 (−5.75, 0.08)	0.018
LCBI, full region of interest	−13.5 (−27.0, −0.06)	15.4 (−1.10, 31.9)	7.29 (−10.7, 25.3)	−4.0 (−33.7, 25.6)	0.24
$LCBI_{max4mm}$	−28.2 (−71.2, 14.7)	46.3 (0.58, 91.9)	9.29 (−52.5, 71.1)	0.04 (−63.7, 63.8)	0.33

*based on a linear trend test across the four quartiles of Δ LDL-c in a linear regression model, with adjustment for age, sex, diabetes, smoking, previous use of statins, and time to recatheterisation. LCBI: lipid core burden index; $LCBI_{max4mm}$: 4 mm segment with the highest LCBI; Δ LDL-c: change in low-density lipoprotein cholesterol (follow-up – baseline); the 25^{th}, 50^{th} and 75th percentile of the Δ LDL-c distribution were −51, −26 and −9 mg/dl, respectively; NC: necrotic core

Online Table 3. Study endpoints by quartiles of baseline values.

Study endpoint	25th, 50th, 75th percentiles of baseline values	Mean change (95% CI)				*p*-value for trend¶
		Baseline Q1	Baseline Q2	Baseline Q3	Baseline Q4	
LDL-c, mmol/l	1.92, 2.36, 2.99	−0.24 (−0.43, −0.06)	−0.37 (−0.60, −0.14)	−0.62 (−0.87, −0.37)	−1.82 (−2.06, −1.57)	<0.001
Atheroma volume, mm^3	143, 204, 305	11.0 (5.83, 16.3)	4.00 (−3.79, 11.8)	2.01 (−3.75, 7.77)	−1.57 (−14.5, 11.4)	0.19
Percent atheroma volume*, %	32.9, 41.5, 48.8	2.35 (1.28, 3.42)	1.69 (0.58, 2.79)	0.19 (−0.84, 1.23)	−0.44 (−1.58, 0.70)	0.001
NC volume, mm^3	7.3, 17.8, 38.0	0.54 (−0.35, 1.42)	−0.15 (−1.97, 1.66)	1.44 (−1.15, 4.03)	−7.45 (−12.4, −2.46)	<0.001
NC percentage, %	15.2, 20.0, 25.0	1.67 (−0.32, 3.67)	−0.08 (−1.48, 1.32)	−2.38 (−4.20, −0.57)	−4.61 (−6.69, −2.51)	<0.001
LCBI, full region of interest	6, 33, 67	19.3 (7.7, 30.9)	16.3 (4.4, 28.2)	1.81 (−12.6, 16.2)	−31.7 (−61.7, −1.7)	0.001
LCBImax4mm	60, 183, 319	83.6 (40.6, 126.6)	46.6 (0.2, 93.0)	−26.1 (−76.0, 23.7)	−82.7 (−138.7, −26.7)	<0.001

¶ based on a linear trend test across the four quartiles in a linear regression model, with adjustment for age, sex, diabetes, smoking, previous use of statins, and time to recatheterisation. * None of the interrogated segments represented a percent atheroma volume ≥70%. CI: confidence interval; LCBI: lipid core burden index; $LCBI_{max4mm}$: 4 mm segment with the highest LCBI; LDL-c: low-density lipoprotein cholesterol; NC: necrotic core

Online Table 4. Serial cholesterol measurements.

	Baseline		Follow-up		Change		
	mean (SD)	median (IQR)	mean (SD)	median (IQR)	mean (95% CI)	*p*-value¶	*p*-value‡
Patients with follow-up IVUS at 6 months (n=30)							
LDL-c, mmol/l	2.34 (0.75)	2.28 (1.92, 2.92)	1.67 (0.56)	1.70 (1.31, 1.88)	−0.76 (−1.00, −0.52)	<0.001	0.98
HDL-c, mmol/l	1.11 (0.31)	1.09 (0.86, 1.31)	1.21 (0.39)	1.13 (0.92, 1.58)	0.10 (0.04, 0.16)	0.004	0.52
Total cholesterol, mmol/l	3.89 (0.89)	3.7 (3.2, 4.5)	3.21 (0.73)	3.3 (2.8, 3.6)	−0.67 (−0.94, −0.40)	<0.001	0.47
Patients with follow-up IVUS at 12 months (n=134) *							
LDL-c, mmol/l	2.51 (0.87)	2.37 (1.91, 3.00)	1.74 (0.74)	1.59 (1.22, 2.02)	−0.77 (−0.94, −0.60)	<0.001	
HDL-c, mmol/l	1.11 (0.31)	1.07 (0.91, 1.28)	1.24 (0.37)	1.19 (0.98, 1.44)	0.13 (0.08, 0.18)	<0.001	
Total cholesterol, mmol/l	4.16 (0.93)	4.0 (3.5, 4.6)	3.36 (0.89)	3.2 (2.7, 3.8)	−0.80 (−0.99, 0.61)	<0.001	
All patients (N=164) *							
LDL-c, mmol/l	2.49 (0.85)	2.36 (1.92, 2.99)	1.73 (0.71)	1.60 (1.26, 2.01)	−0.76 (−0.91, −0.61)	<0.001	
HDL-c, mmol/l	1.11 (0.31)	1.07 (0.90, 1.29)	1.23 (0.37)	1.18 (0.97, 1.46)	0.12 (0.08, 0.16)	<0.001	
Total cholesterol, mmol/l	4.11 (0.93)	4.0 (3.3, 4.6)	3.34 (0.87)	3.3 (2.7, 3.8)	−0.77 (−0.93, −0.61)	<0.001	

* Six patients had missing baseline and/or follow-up measurements. ¶ based on linear mixed models (patient as random intercept) to test if change is different from 0. ‡based on two-sample Student's t-tests (equal variances not assumed) for the difference in change between patients with 6 versus 12 months of follow-up. CI: confidence interval; HDL-c: high-density lipoprotein cholesterol; IQR: interquartile range; LDL-c: low-density lipoprotein cholesterol; SD: standard deviation

Online Table 5. Serial intravascular ultrasound measurements.

	Baseline		Follow-up		Change		
	mean (SD)	median (IQR)	mean (SD)	median (IQR)	mean (95% CI)	*p*-value¶	*p*-value‡
Patients with follow-up IVUS at 6 months (n=30)							
External elastic membrane volume, mm^3	560.4 (278.2)	495.7 (345.7, 724.9)	548.1 (255.7)	510.8 (344.1, 713.2)	−12.3 (−25.8, 1.2)	0.083	0.42
Lumen volume, mm^3	321.4 (150.6)	280.6 (192.7, 438.8)	316.3 (139.7)	292.1 (196.6, 407.8)	−5.1 (−16.0, 5.8)	0.37	0.015
Atheroma volume, mm^3	239.1 (144.4)	200.2 (137.7, 304.1)	231.8 (132.4)	193.6 (139.6, 287.2)	−7.2 (−16.5, 2.0)	0.12	0.010
Percent atheroma volume, %	41.7 (8.3)	43.6 (33.3, 47.0)	41.5 (8.5)	42.9 (33.2, 48.9)	−0.2 (−1.2, 0.8)	0.68	0.018
NC volume, mm^3	23.2 (25.6)	16.2 (8.7, 35.3)	21.5 (25.5)	13.8 (5.7, 31.4)	−1.7 (−4.1, 0.8)	0.18	0.84
DC volume, mm^3	9.1 (9.2)	6.6 (2.0, 12.2)	8.9 (8.8)	6.3 (2.2, 12.3)	−0.2 (−1.2, 0.9)	0.75	0.32
FI volume, mm^3	71.4 (66.4)	50.1 (32.7, 87.7)	66.4 (57.9)	53.1 (28.7, 82.6)	−5.0 (−10.2, 0.2)	0.058	0.051
FF volume, mm^3	16.0 (16.8)	10.1 (4.5, 21.8)	15.8 (17.8)	9.7 (5.4, 21.8)	−0.3 (−3.8, 3.3)	0.88	0.15
NC percentage, %	18.1 (5.6)	18.6 (14.9, 22.4)	17.2 (6.1)	18.1 (13.3, 21.0)	−0.9 (−2.4, 0.6)	0.22	0.52
DC percentage, %	7.7 (4.5)	6.7 (4.2, 10.2)	7.8 (4.4)	7.2 (4.8, 10.8)	0.0 (−0.8, 0.9)	0.92	0.99
FI percentage, %	61.6 (8.9)	63.9 (57.9, 68.0)	61.8 (9.0)	63.3 (56.4, 66.7)	0.3 (−1.5, 2.0)	0.76	0.13
FF percentage, %	12.6 (4.5)	12.9 (8.9, 15.4)	13.2 (5.7)	12.6 (9.9, 15.5)	0.6 (−1.0, 2.2)	0.44	0.048
Patients with follow-up IVUS at 12 months (n=134)							
External elastic membrane volume, mm^3	583.9 (278.8)	527.6 (384.2, 754.1)	583.5 (277.7)	521.5 (381.7, 718.1)	−0.6 (−8.1, 6.9)	0.87	
Lumen volume, mm^3	338.5 (149.9)	325.5 (231.8, 409.1)	332.1 (147.5)	316.1 (227.3, 398.9)	−7.0 (−13.0, −1.0)	0.025	
Atheroma volume, mm^3	245.0 (153.3)	204.0 (144.0, 304.8)	251.4 (152.2)	214.0 (152.2, 312.2)	6.4 (1.8, 10.9)	0.006	
Percent atheroma volume, %	40.5 (10.7)	40.5 (32.5, 49.0)	41.7 (10.0)	41.4 (33.9, 50.1)	1.2 (0.6, 1.9)	<0.001	
NC volume, mm^3	30.5 (33.1)	19.9 (7.2, 43.3)	29.1 (32.3)	20.1 (6.3, 37.1)	−1.4 (−3.2, 0.5)	0.14	
DC volume, mm^3	13.9 (17.0)	7.9 (2.4, 19.3)	14.4 (18.1)	8.7 (2.2, 18.1)	0.5 (−0.4, 1.5)	0.27	
FI volume, mm^3	71.1 (63.6)	51.3 (30.9, 94.2)	71.8 (62.8)	52.8 (31.0, 95.0)	0.8 (−2.0, 3.6)	0.57	
FF volume, mm^3	13.2 (14.1)	8.5 (3.5, 17.8)	15.7 (14.7)	10.9 (5.4, 23.3)	2.5 (1.0, 4.0)	0.001	
NC percentage, % *	20.7 (8.7)	20.8 (16.2, 25.7)	19.3 (7.6)	20.1 (15.2, 24.4)	−1.5 (−2.6, −0.4)	0.012	

Online Table 5 *(continued)*

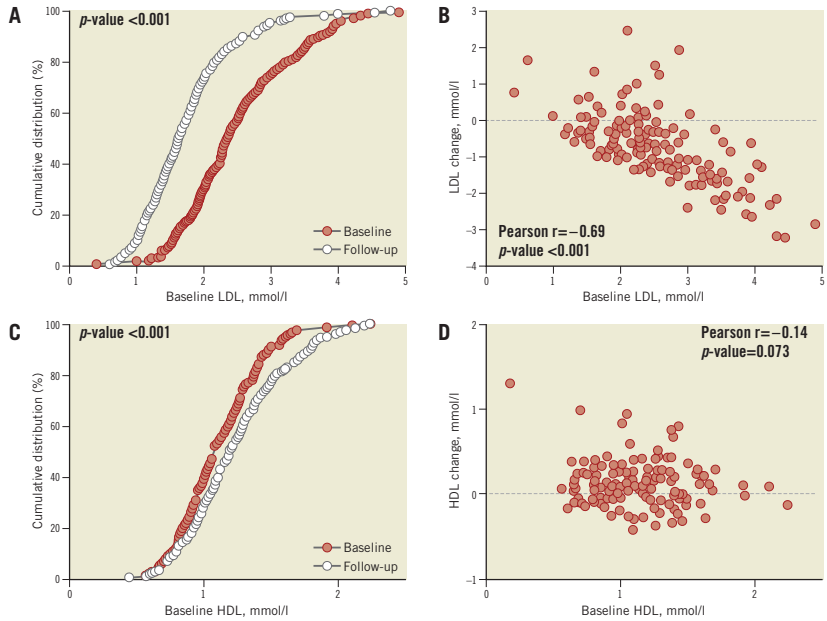
	Baseline		Follow-up		Change		
	mean (SD)	**median (IQR)**	**mean (SD)**	**median (IQR)**	**mean (95% CI)**	***p*-value¶**	***p*-value‡**
DC percentage, % *	9.3 (5.8)	8.7 (4.9, 13.0)	9.5 (6.2)	8.9 (4.2, 13.4)	0.1 (−0.8, 0.9)	0.86	
FI percentage, % *	59.7 (11.4)	59.4 (51.9, 66.5)	58.0 (11.3)	59.2 (50.4, 65.5)	−1.5 (−3.2, 0.1)	0.058	
FF percentage, % *	10.3 (5.3)	9.4 (7.3, 13.0)	13.2 (10.6)	11.8 (8.1, 15.6)	3.0 (1.2, 4.8)	0.002	
All patients (N=164)							
Atheroma volume, mm^3	243.9 (151.3)	204.0 (142.7, 304.8)	247.8 (148.6)	210.9 (145.4, 301.8)	3.9 (−0.2, 8.0)	0.064	
Percent atheroma volume, %	40.7 (10.2)	41.5 (32.9, 48.8)	41.6 (9.7)	41.5 (33.8, 49.8)	1.0 (0.4, 1.5)	0.001	
NC volume, mm^3	29.1 (31.9)	17.8 (7.3, 38.0)	27.7 (31.2)	19.2 (6.2, 35.1)	−1.4 (−3.0, 0.1)	0.074	
DC volume, mm^3	13.0 (15.9)	7.9 (2.3, 17.4)	13.4 (16.9)	8.2 (2.2, 17.2)	0.4 (−0.4, 1.2)	0.31	
FI volume, mm^3	71.1 (63.9)	51.3 (31.1, 93.4)	70.8 (61.8)	52.8 (30.6, 94.6)	−0.3 (−2.7, 2.2)	0.83	
FF volume, mm^3	13.7 (14.6)	9.0 (3.9, 18.8)	15.7 (15.3)	10.9 (5.4, 22.1)	2.0 (0.6, 3.4)	0.005	
NC percentage, % *	20.2 (8.2)	20.0 (15.2, 25.0)	18.9 (7.3)	19.5 (14.6, 24.0)	−1.4 (−2.4, −0.4)	0.006	
DC percentage, % *	9.0 (5.6)	8.4 (4.6, 12.7)	9.1 (5.9)	8.4 (4.4, 13.1)	0.0 (−0.6, 0.7)	0.85	
FI percentage, % *	60.0 (11.0)	60.5 (52.6, 66.8)	58.7 (11.0)	60.7 (50.6, 66.2)	−1.2 (−2.6, 0.2)	0.076	
FF percentage, % *	10.7 (5.2)	9.9 (7.5, 13.7)	13.2 (9.9)	11.8 (8.5, 15.6)	2.6 (1.1, 4.1)	0.001	

*One patient had missing follow-up measurements. ¶ based on linear mixed models (patient as random intercept) to test if change is different from 0. ‡ based on two-sample Student's t-tests (equal variances not assumed) for the difference in change between patients with 6 versus 12 months of follow-up. CI: confidence interval; DC: dense calcium tissue; FF: fibro-fatty tissue;
FI: fibrous tissue; IQR: interquartile range; NC: necrotic core tissue; SD: standard deviation

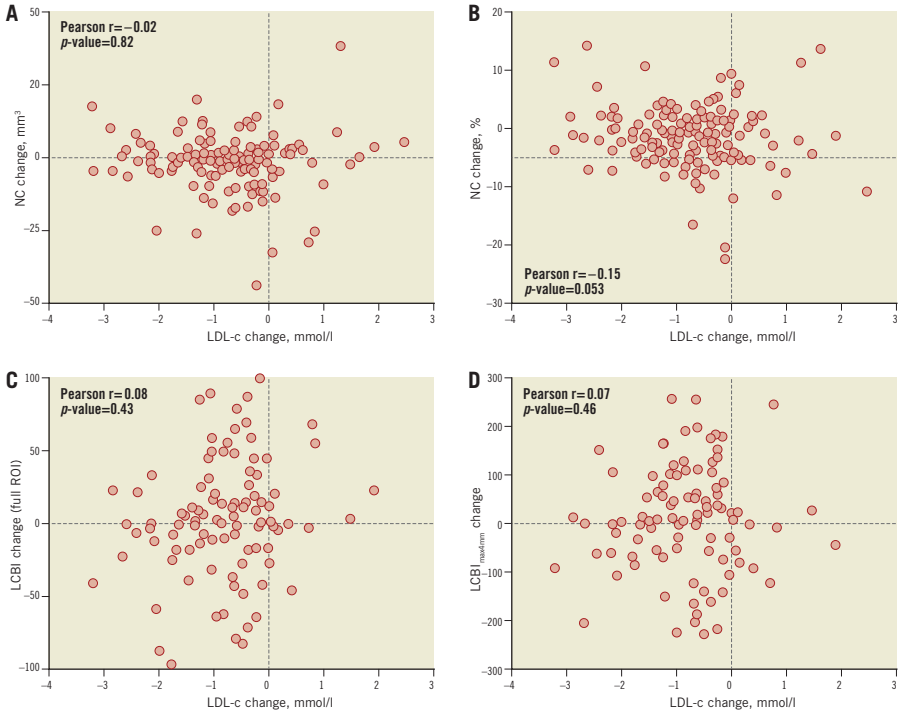
Online Table 6. Serial near-infrared spectroscopy measurements.

	Baseline		Follow-up		Change		
	mean (SD)	median (IQR)	mean (SD)	median (IQR)	mean (95% CI)	*p*-value¶	*p*-value‡
Patients with follow-up NIRS at 6 months (n=26)							
LCBI, full region of interest	48.6 (42.5)	40.0 (10.0, 67.0)	45.0 (39.8)	40.0 (7.0, 72.0)	−3.6 (−22.2, 15.1)	0.70	0.55
LCBI, worst 10 mm	141.9 (123.0)	147.0 (29.0, 201.0)	131.2 (111.2)	128.5 (29.0, 188.0)	−10.7 (−54.9, 33.4)	0.62	0.46
LCBI, worst 4 mm	220.2 (157.4)	242.5 (69.0, 310.0)	206.5 (157.7)	201.0 (60.0, 305.0)	−13.7 (−68.3, 40.9)	0.61	0.42
Patients with follow-up NIRS at 12 months (n=77)							
LCBI, full region of interest	43.6 (53.9)	28.0 (5.0, 63.0)	46.5 (44.6)	35.0 (9.0, 66.0)	2.8 (−8.8, 14.5)	0.63	
LCBI, worst 10 mm*	123.0 (121.7)	98.0 (21.5, 189.0)	130.2 (115.7)	104.5 (36.5, 196.0)	7.2 (−14.8, 29.3)	0.52	
LCBI, worst 4 mm*	195.6 (166.5)	174.5 (52.5, 324.0)	206.9 (154.5)	190.0 (77.0, 324.0)	11.2 (−19.7, 42.2)	0.47	
All patients (N=103)							
LCBI, full region of interest	44.9 (51.1)	33.0 (6.0, 67.0)	46.1 (43.2)	35.0 (8.0, 72.0)	1.2 (−8.5, 11.0)	0.80	
LCBI, worst 10 mm*	127.8 (121.7)	107.0 (25.0, 197.0)	130.5 (114.0)	109.0 (30.0, 194.0)	2.7 (−16.9, 22.2)	0.79	
LCBI, worst 4 mm*	201.9 (163.8)	182.5 (60.0, 319.0)	206.8 (154.5)	192.0 (72.0, 323.0)	4.9 (−21.7, 31.4)	0.72	

*One patient had missing follow-up measurements. ¶based on linear mixed models (patient as random intercept) to test if change is different from 0. ‡ based on two-sample Student's t-tests ssumed) for the difference in change between patients with 6 versus 12 months of follow-up. CI: confidence interval; IQR: interquartile range; LCBI: lipid core burden index; SD: standard deviation



Online Figure 1. Serum cholesterol levels at baseline and follow-up.
High-intensity rosuvastatin therapy during a median follow-up of 372 days resulted in a significant decrease in serum LDL-c (A) and increase in HDL-c levels (C), despite the fact that 95% of the patients were already on standard-of-care statin therapy at baseline. The degree of reduction in LDL-c was related to the baseline LDL-c level (B). Such a correlation was not observed with respect to HDL-c (D). HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol



Online Figure 2. Change in LDL-c in relation to change in necrotic core and LCBI.
Changes in NC and LCBI were independent of changes in LDL levels under rosuvastatin therapy. A) NC volume; B) NC percentage; C) Full region of interest; D) $LCBI_{max4mm}$. LCBI: lipid core burden index; $LCBI_{max4mm}$: the 4 mm segment with the highest LCBI; LDL-c: low-density lipoprotein cholesterol; NC: necrotic core

CORONARY VULNERABILITY

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PREDICTION OF ABSOLUTE RISK REDUCTION OF CARDIOVASCULAR EVENTS WITH PERINDOPRIL FOR INDIVIDUAL PATIENTS WITH STABLE CORONARY ARTERY DISEASE – RESULTS FROM EUROPA

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ABSTRACT

Background: Angiotensin-converting-enzyme inhibition reduces the risk of cardiovascular events at a group level. Presumably, the absolute effect of treatment varies between individuals. We sought to develop multivariable prediction scores to estimate individual treatment effect of perindopril in patients with stable coronary artery disease (sCAD).

Methods: In EUROPA trial participants, we estimated the individual patient 5-year absolute risk reduction (ARR) of major adverse cardiovascular events (MACE) by perindopril. Predictions were based on a new Cox proportional-hazards model with clinical characteristics and an external risk score in combination with the observed relative risk reduction. Second, a genetic profile modifying the relative efficacy of perindopril was added. The individual patient ARR was defined as the difference in MACE risk with and without treatment. The group level impact of selectively treating patients with the largest predicted treatment effect was evaluated using net benefit analysis.

Results: The risk score combining clinical and genetic characteristics estimated the 5-year absolute treatment effect to be absent or adverse in 27% of patients. On the other hand, the risk score estimated a small 5-year ARR of ≤2% ($NNT_5 \geq 50$) in 20% of patients, a modest ARR of 2–4% (NNT_5 25–50) in 26%, and a large ARR of ≥4% ($NNT_5 \leq 25$) in 28%. The external risk score yielded similar predictions. Selective prediction-based treatment resulted in higher net benefit compared to treat everyone at any treatment threshold.

Conclusion: A prediction score combining clinical characteristics and genetic information can quantify the ARR of MACE by perindopril for individual patients with sCAD and may be used to guide treatment decisions.

Trial registration number: ISRCTN37166280

1. INTRODUCTION

Activation of the renin–angiotensin system (RAS) has an important role in the development of cardiovascular disease [1]. The beneficial effects of blocking RAS by angiotensin-converting-enzyme inhibitors (ACE-i) were first demonstrated in patients with heart failure [2,3]. Further studies showed the efficacy of ACE-i in a wider range of clinical conditions and these agents are currently recommended for the treatment of patients with hypertension, recent myocardial infarction and stable coronary artery disease (sCAD) [4]. One of the landmark trials in patients with sCAD is the EUROPA trial, evaluating the effect of perindopril on the occurrence of new major adverse cardiovascular events (MACE) in sCAD patients without heart failure. The trial found an average relative reduction in MACE of 20% [5]. The average 4-year absolute treatment effect of perindopril was 2%, translating to an average 4-year number-needed-to-treat (NNT_4) of 50 patients to prevent one event [5]. In search of patients who are most likely to benefit, stratified analyses based on clinical characteristics and levels of baseline risk revealed similar relative risk reductions across all subgroups [6,7]. However, genetic variations in pharmacodynamic pathways affected by ACE-i were shown to influence the efficacy of perindopril [8]. Three polymorphisms located in the angiotensin-II type 1 receptor and bradykinin type 1 receptor genes were associated with a larger, smaller or even adverse effect of perindopril. To quantify the effect of treatment for individual patients, relative risk reductions need to be interpreted in combination with absolute event risks [9–12]. In general, patients with higher baseline risk tend to benefit more from treatment in terms of absolute risk reduction [13,14]. Baseline risk is determined by the combined action of multiple risk factors such as age, cholesterol and blood pressure [15]. In the present study we sought to develop prediction scores based on a combination of multiple patient-specific clinical and genetic characteristics to estimate the absolute risk reduction of MACE with perindopril for individual patients with sCAD. In clinical practice, these scores can be used to quantify treatment benefit at an individual patient level and to guide treatment decisions.

2. METHODS

The design, rationale and outcomes of the EUROPA trial and the PERindopril GENetic association study (PERGENE) substudy have been described elsewhere [16,17]. Briefly, the EUROPA trial was a randomized, double blind study evaluating the effect of perindopril 8mg once daily versus placebo on major cardiovascular adverse events (MACE) comprising cardiovascular death, myocardial infarction (MI), and resuscitated cardiac arrest in 12,218 patients with sCAD. Eligible patients were men and women of 18 years or older,

with evidence of coronary heart disease documented by previous MI (>3 months before screening), percutaneous or surgical coronary revascularization (>6 months before screening), angiographic evidence of at least 70% narrowing of at least one major coronary artery, or a history of typical chest pain in male patients with an abnormal stress test. Exclusion criteria included clinically evident heart failure, planned revascularization procedure, hypotension (sitting systolic blood pressure <110 mm Hg), uncontrolled hypertension (systolic blood pressure >180 mm Hg and/or diastolic blood pressure >100 mm Hg), use of ACE-i or angiotension-2 receptor blockers in the last month, renal insufficiency (serum creatinine >150 µmol/L), and serum potassium >5.5mmol/L. PERGENE is a substudy of the EUROPA trial designed to investigate whether common genetic variation is related to risk of future events and modifies the treatment effect of perindopril [8]. Blood samples were received from 10060 patients and 8726 patients had complete genotype data on rs275651, rs5182 and rs12050217, the three single nucleotide polymorphisms (SNPs) identified to modify the effect of perindopril [8, 18]. A genetic profile was constructed by counting the number of unfavorable alleles and grouping them into 3 categories: ≤1 unfavorable allele (reference), 2 unfavorable alleles and ≥3 unfavorable alleles. Approval for the trial was obtained from the institutional ethics committee of each center and all participants provided written informed consent.

2.1. Model derivation

The individual patient absolute treatment effect on MACE was estimated with clinical models and with models combining clinical and genetic characteristics. First, we fitted a new Cox proportional hazards model (i.e. EUROPA score) based on a set of clinical characteristics together with a treatment variable (placebo vs. active treatment) [7]. The prespecified predictors were: sex, age, systolic blood pressure, cholesterol, body-mass index (BMI), diabetes, smoking, estimated glomerular filtration rate (eGFR; by CKD-EPI equation [19]), symptomatic CAD, family history of CAD, prior stroke or transient ischemic attack, prior MI, prior coronary revascularization and prior peripheral arterial disease and treatment status. Restricted cubic splines were used to assess the linearity assumption for continuous predictors. If the association between a continuous predictor and the outcome was not linear, the predictor was transformed to improve model fit [20,21]. As a result, age, BMI and eGFR were included both as linear and squared terms. We used the Lasso method (i.e. penalized partial maximum likelihood with a restriction on the sum of the absolute coefficients of standardized predictors) to select the model and shrink the model coefficients to minimize over-optimism [22,23]. The interaction between treatment and baseline risk was evaluated but not significant [24]. The model was fitted for the prediction of 4.3-year (median follow-up) risks and extrapolated to yield 5-year estimates. The individual patient absolute risk reduction (ARR) was defined as the difference between estimated on-treatment and off-treatment risk.

Second, we evaluated the combination of clinical and genetic characteristics to predict absolute treatment effect for individual patients. Hereto, we expanded the EUROPA model with a genetic profile (3-level categorical variable) and the interaction between perindopril treatment and this profile (i.e. EUROPA-GEN score). Again, the Lasso method was used to select the model and shrink the coefficients. The clinical model was fitted in the full EUROPA cohort, whereas the model with additional genetic variables was fitted in the PERGENE subsample. All models were evaluated in the PERGENE subsample to ensure comparability of results.

Supplementary analyses encompassed the use of an externally developed risk algorithm, the SMART risk score, together with the relative treatment effect observed in the trial [25]. The baseline risk of the SMART risk score was recalibrated to the 5-year disease incidence of the target population. Data on HDL cholesterol, high sensitivity C-reactive protein and history of abdominal aortic aneurysm were not available and were set to zero. In addition, the genetic profile and the treatment interaction of perindopril with this profile were added to this model. The Lasso method was used to select and shrink the newly added variables.

Data was missing in 10.1% of participants for the variable 'years since first vascular event' and in <1% for all other variables. Missing data were reduced by single imputation methods using predictive mean matching [26].

2.2. Model performance

Discrimination of the risk scores was assessed by calculation of Harrell's c-statistic [21]. Calibration of predicted risk was assessed by plotting observed 4.3-year event free survival against the average predicted 4.3-year event free survival within deciles and was formally checked by the Gronnesby and Borgan test [27,28]. Since the actual interest was the accuracy of predicted ARR rather than risk, we also assessed whether predicted ARR was in agreement with observed ARR by comparing observed survival within quintiles of patients with similar estimated ARR from the placebo and intervention group. Optimally, the observed survival difference between these paired quintiles should be similar to the estimated ARR.

2.3. Distribution of absolute treatment effect and net benefit

The distributions of predicted individual 5-year ARR of MACE were displayed in histograms. Next, we evaluated the incremental value of applying therapeutic prediction models in clinical practice using the net benefit method [29]. The calculation of net benefit is based on the weighing of positive and negative effects of treatment. The severity of treatment disadvantages is expressed relative to the outcome by a threshold NNT. For example, a 5-year threshold NNT of 50 implies that the disadvantages of treating 50 patients for 5 years are considered to be well balanced by the benefit obtained

by preventing one outcome. Net benefit is calculated as the observed ARR in patients for whom the treatment recommended by the prediction algorithm is congruent with randomized allocation minus the disadvantages of treatment. The latter is defined as the proportion of patients treated weighted by the inverse of the threshold NNT (net benefit = ARR – proportion of patients treated * [1/threshold NNT]). Net benefit can be interpreted as the excess number of events prevented per 100 patients on top of the minimally required number of events prevented to offset treatment disadvantages. We considered the following treatment strategies; (i) treat no one, (ii) treat everyone or (iii) prediction-based treatment (i.e. selective treatment of patient whose predicted treatment effect exceeds the specified threshold NNT). Lastly,we showed the impact of using a prediction-based treatment strategy in clinical practice. Statistical analyses were conducted in R, version 2.15.2 (RDevelopment Core Team, Vienna, Austria) with Harrell's Regression Modelling Strategies package and Goeman's 'penalized' package.

3. RESULTS

Baseline characteristics of the PERGENE participants (n = 8726) are shown in Table 1 and are similar to those of the whole EUROPA population. During a median follow-up of 4.3 years 794 major cardiovascular events occurred. The hazard ratio of the overall treatment effect for MACE was 0.80 (95% CI 0.71–0.91) favoring treatment with perindopril.

3.1. Model derivation & performance

3.1.1. Clinical model

The EUROPA models are presented in Box 1. All predictors in the EUROPA model were retained. Detailed statistics are presented in Supplement Table 1. Discrimination was moderate with a c-statistic of 0.67 [95% CI 0.65–0.69]. The EUROPA model showed good risk calibration (*p*-value 0.34) (Supplement Fig. 1). The ARR calibration plot showed an acceptable agreement between predicted and observed ARR (Fig. 1). The externally developed SMART model is shown in Supplement Box 1.

3.1.2. Clinical model combined with genetic profile

During model selection all clinical, genetic and interaction variables were retained in the EUROPA-GEN score (Box 1). Detailed statistics are available in Supplement Table 1. Discrimination was moderate with a c-statistic of 0.68 [95% CI 0.66–0.70]. The EUROPA-GEN models showed good visual calibration (although contradicted by a *p*-value of 0.01 by the formal test statistics) (Supplement Fig. 1). Notably, the ARR calibration plot of the expanded model showed a wider range of predicted and observed treatment effects.

Table 1. Baseline characteristics of 8726 EUROPA participants with available genetic profile and stratified according to predicted 5-year absolute risk reduction (ARR) by the EUROPAGEN score.

	Total population (n = 8726)	<2% ARR (n =4077)	≥2% ARR (n= 4649)
Clinical characteristics			
Age (years)	59.8 (9.3)	58.8 (8.9)	60.7 (9.5)
Gender, % female	14.5	17.3	12.0
Hypertension, %	28.5	27.2	29.7
Diabetes, %	12.7	8.9	16.1
Current smoking, %	14.7	12.8	16.5
Duration of vascular disease, years	4.3 (4.7)	3.9 (4.3)	4.6 (4.9)
Body mass index (kg/m^2)	27.5 (3.5)	27.3 (3.2)	27.6 (3.7)
Symptomatic CAD†, %	25.4	19.8	30.4
Family history of CAD, %	27.2	27.2	27.2
Prior myocardial infarction, %	65.4	59.9	70.1
Prior revascularization, %	54.6	59.7	50.1
Prior stroke or TIA, %	3.5	2.6	4.4
Prior PVD, %	7.4	5.1	9.4
Total cholesterol (mmol/L)	5.4 (1.0)	5.3 (1.0)	5.5 (1.1)
eGFR (mL/min/1.73 m^2)	75 (64–87)	77 (66–89)	73 (62–86)
Randomized treatment, %	49.7	50.4	49.1
Systolic blood pressure (mm Hg)	137 (15)	136 (15)	138 (15)
Diastolic blood pressure (mm Hg)	82 (8)	82 (8)	82 (8)
Genetic profile			
≤1 unfavorable allele, %	41.1	2.7	74.7
2 unfavorable alleles, %	32.4	40.5	25.3
≥3 unfavorable alleles, %	26.5	56.8	0

Summary statistics for continuous variables are presented as mean (standard deviation) or as median (interquartile range). Categorial variables are presented as precentages.
ARR: absolute risk reduction. eGFR: estimated glomerular filtration rate estimated by CKD-EPI equation, LDL: low density lipoprotein, HDL: high density lipoprotein, TIA: transient ischemic attack, PVD: peripheral vascular disease.
† Agina pectoris or previous heart failure.

The agreement between predicted and observed absolute treatment effect was generally close (Fig. 1). The SMART-GEN model is shown in Supplement Box 1 and detailed model statistics are presented in Supplement Table 1.

3.2. Distribution of treatment effect of perindopril

The EUROPA score predicted a small 5-year ARR ≤2% (NNT_5≥50) in 60.1% of patients. The predicted 5-year ARR was between 2 and 4% (NNT_5 25–50) in 33.5% of patients and ≥4%

Box 1. The EUROPA risk scores

Individual patient off–treatment risk : "treatment" = 0 (NO)
Individual patient on–treatment risk : "treatment" = 1 (YES)

A) EUROPA score

5–year MACE risk (%) = (1 – 0.91$^{exp(A + 6.415)}$) × 100%
A = age * –0.1324 + age^2 * 0.0013 + female sex * –0.4643 + SBP * 0.0041 + total cholesterol * 0.1499 + eGFR eGFR *–0.0339 + eGFR^2 * 0.0002 + BMI *–0.2590 + BMI^2 * 0.0049 + diabetes * 0.4481 + current smoking * 0.3876 + family history of CAD * 0.1662 + prior MI * 0.3671 + prior TIA or stroke * 0.4446 + prior PVD * 0.5092 + prior coronary revascularization * –0.2235 + symptomatic CAD * 0.3981 + treatment *–0.2167

B) EUROPA-GEN score

5–year MACE risk (%) = (1 – 0.91$^{exp(A + 7.390)}$) × 100%
A = age * –0.1351 + age^2 * 0.0013 + female sex * –0.5474 + SBP * 0.0040 + total cholesterol * 0.1237 + eGFR eGFR *–0.0358 + eGFR^2 * 0.0002 + BMI *–0.2958 + BMI^2 * 0.0056 + diabetes * 0.4673 + current smoking * 0.4449 + family history of CAD * 0.0791 + prior MI * 0.4055 + prior TIA or stroke * 0.4433 + prior PVD * 0.5340 + prior coronary revascularization *–0.1582 + symptomatic CAD * 0.4080 + genetic profile 1 *–0.2062 + genetic profile 2 *–0.5112 + treatment * –0.5466 + treatment & 2 unfavorable alleles * 0.3207 + treatment & ≥3 unfavorable alleles * 0.7498

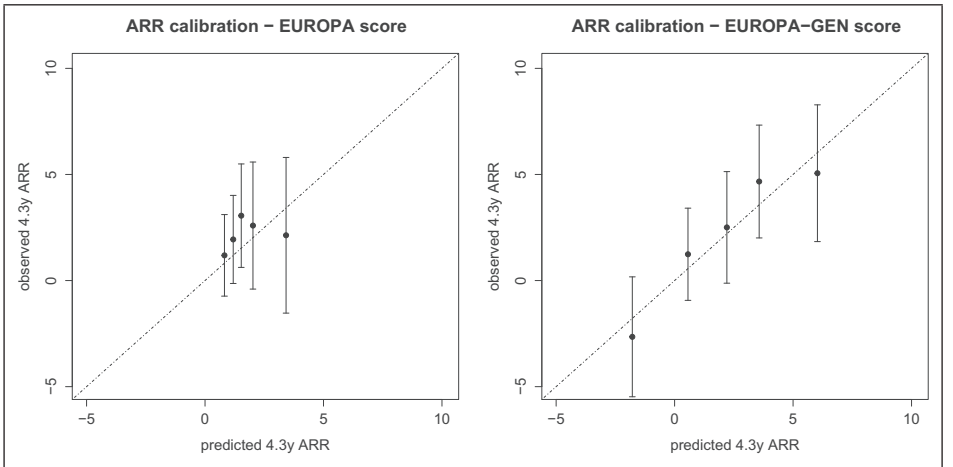

Fig. 1. Calibration plots of predicted versus observed 4.3 year absolute risk reduction (ARR) of major cardiovascular events (MACE) in quintiles for different prediction scores.

(NNT$_5$ ≤ 25) in 6.4% of patients (Fig. 2). The SMART score identified similar proportions of patients in these categories of absolute treatment effect (Supplement Fig. 2).

The EUROPA-GEN score predicted an absent or adverse treatment effect in 26.5% of patients (Fig. 2). These adverse responders were characterized by an unfavorable genetic profile (i.e. ≥3 unfavorable alleles) and were at higher cardiovascular risk when treated with perindopril compared with placebo. Alternatively, the EUROPA-GEN score predicted a large 5-year ARR of ≥4% (NNT$_5$ ≤ 25) in 27.7% of patients. The SMART-GEN score identified similar proportions per category (Supplement Fig. 2). Table 1 displays the characteristics of patients stratified according to predicted treatment effect, showing higher risk factor levels and a skewed genetic profile in patients with a larger ARR.

3.3. Net benefit and clinical consequences of individualized prediction of treatment effect of perindopril

Across the entire range of 5-year treatment threshold NNTs, the clinical EUROPA score was not associated with higher net benefit at a population level compared to treating everyone or no one (Fig. 3). Hence, this model does not succeed in accurately directing treatment to sCAD patients who can anticipate the largest benefit from perindopril. On the other hand, the EUROPA-GEN score showed higher net benefit compared to treating everyone or no one across a wide range of treatment thresholds (Fig. 3). Even if the treatment threshold is infinite, suggesting one is prepared to treat a vast number of patients (e.g. >250) for 5 years to prevent a single event, prediction-based treatment is superior. This could be expected since prediction-based treatment limits prescription to the 73.5% of sCAD patients with an estimated positive effect, while withholding treatment for the 26.5% of patients with an estimated adverse or null effect (Table 2). Results were similar for the SMART risk scores with and without genetic characteristics (Supplement Fig. 3). When restricting treatment to patients with larger predicted treatment effects, the average 5-year NNT among treated patients could be reduced from 42 to 12 depending on the choice of treatment threshold (Table 2).

Table 2. Consequences for clinical practice based on EUROPA-GEN score.

5-year threshold NNT	Tx-strategy	Tx-rate*	5-year average ARR†	5-year average NNT†
–	Treat all	100%	2.4%	42
250	Prediction-based Tx	73%	3.9%	25
100	Prediction-based Tx	70%	4.1%	25
50	Prediction-based Tx	53%	4.9%	20
25	Prediction-based Tx	28%	6.7%	15
17	Prediction-based Tx	13%	8.7%	12

Tx: Treatment. *Percentage of total population treated with perindopril. †Predicted average reduction in absolute risk of MI, resuscitated cardiac arrest and vascular death in selection of patients actively treated with perindopril.

4. DISCUSSION

In the present study we demonstrated that therapeutic prediction models based on clinical and genetic characteristics were able to quantify the ARR of major cardiovascular events by perindopril for individual patients with sCAD. Of all participants, 27% had an absent or adverse treatment effect whereas 28% had a large estimated 5-year ARR of ≥4% ($NNT_5 \leq$ 25). Selective treatment of patients based on a prediction score can result in a more optimal trade-off between the number of events prevented and number of patients treated.

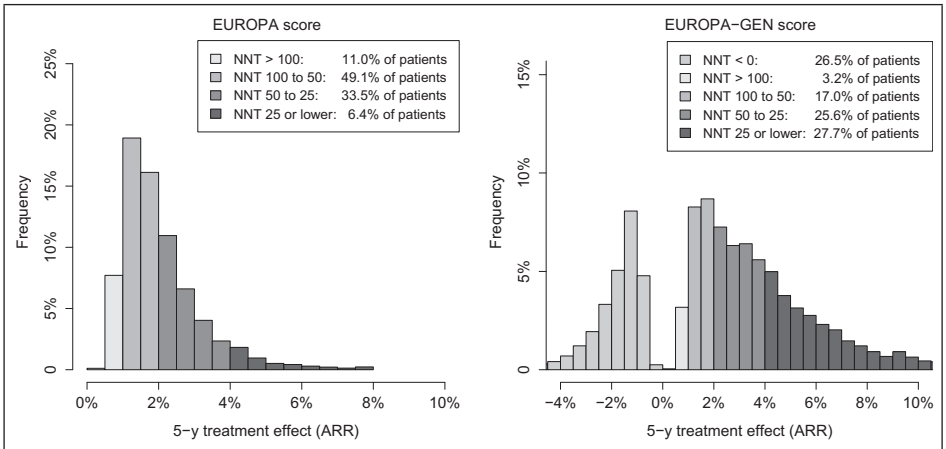

Fig. 2. Distribution of 5-year absolute risk reduction (ARR) of major cardiovascular events with perindopril treatment for individual patients with stable CAD.

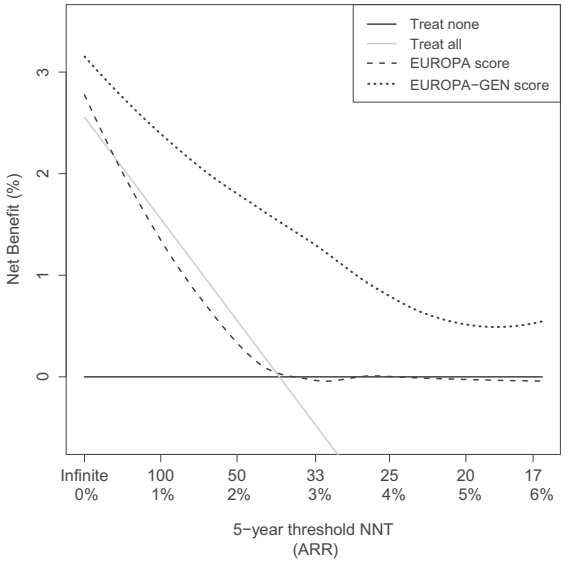

Fig. 3. Net benefit curves of different treatment strategies for major cardiovascular events (MACE). Net benefit is calculated as the observed ARR of MACE (%) in patients whose randomized allocation is similar to recommendations from the treatment score minus the disadvantages of treatment. The disadvantages of treatment are expressed as the proportion of patients receiving treatment weighted by the threshold NNT. First, a (range of) threshold NNT should be determined and next the strategy associated with the highest net benefit for this (range of) threshold can be extracted from the graph. The threshold NNT may vary among clinicians and patients. The treat all line originates at the average ARR observed in the trial, since the negative effects of treatment are assumed to be zero at an infinite threshold NNT_5. Treat none is associated with zero net benefit.

Guidelines recommend ACE-i in patients with sCAD, especially if there are co-existing conditions such as hypertension, reduced left ventricular ejection fraction or chronic kidney disease [30,31]. These recommendations are based on the overall results of large randomized clinical trials showing reductions in cardiovascular events and mortality [5,32, 33]. Even if the relative risk reduction is constant, the absolute risk reduction with treatment varies and is likely to increase with baseline event risk. However, in the present study the relative efficacy of perindopril was influenced by the patients' genetic profile and patients with similar baseline risks had different risk reductions. Consequently, the clinical scores with just a single treatment variable (i.e. active vs. placebo)were unable to accurately pinpoint the expected individual patient treatment benefit as was illustrated by the weak relation between predicted and observed ARR. Conversely, the prediction scores with both clinical and genetic variables combined the patient's baseline risk with an efficacy measure (i.e. hazard ratio) that was applicable to the patient's specific genetic profile and yielded accurate estimates of individual treatment effect. The patient-specific ARR can be translated to an individualized NNT (iNNT),which refers to the number of patients with the same characteristics as the patient under care that require treatment for a specific time to prevent one event (Box 3)[11].

The effect of implementing an individualized treatment strategy in clinical practice was evaluated at a group level [29]. Selective drug prescription, based on an individualized treatment prediction algorithm, can direct treatment to those patients who might expect the largest benefit and least harm of treatment. The choice of an appropriate treatment threshold is difficult as the threshold comprises adverse effects of the drug, the inconvenience of daily taking a drug and monetary costs. Notably, the frequency of adverse effects is difficult to estimate based on trial results as only patients who tolerated perindopril were randomized after a run-in period. For randomized patients, the difference in adherence to allocated therapy was 3.4% in the EUROPA [5]. Hence, treating for example 100 patients in clinical practice will result in at least 3 patients experiencing an adverse effect prompting them to discontinue the drug. Secondly,

Box 3. Predicted 5-year absolute risk reduction of MACE when treated with perindopril for two different patient profiles.
Patient A
A asymptomatic 60-year old non-smoking male patient without diabetes, an SBP of 130mm Hg, a BMI of 25 kg/m^2, no family history of CAD, no prior MI, no prior stroke, no prior PVD, a CABG procedure 2 years ago, a TC of 6 mmol/L, an eGFR of 60 ml/min and 2 unfavorable alleles. → 5-year ARR with perindopril is 1.1% (individual NNT_5=88)
Patient B
Asymptomatic 60-year old smoking male patient with diabetes, an SBP of 150 mm Hg, a BMI of 30 kg/m^2, no family history of CAD, no prior MI, no prior stroke, no prior PVD, no prior revascularization, a TC of 6mmol/L, an eGFR of 60ml/min and ≤1 unfavorable allele. → 5-year ARR with perindopril is 11.6% (individual NNT_5=9)

disadvantages include the inconveniences of 100 patients who need to take perindopril daily for 5 years (i.e. 500 person-years of treatment). Thirdly, there are economic costs of perindopril prescription. At a 5-year threshold NNT of 100, we consider all the negative effects of treating 100 patients together to be balanced by the prevention of, for example, one MACE. We acknowledge that this summary of positive and negative effects is incomplete and subject to interpretation. Therefore, we specified a range of treatment thresholds, defined as the number of patients one would be willing to treat to prevent one adverse cardiovascular outcome, to allow clinicians and patients to make their own appraisal of treatment risks and benefits. Further, the treatment threshold may change over time as for example drug costs decrease. A prediction-based treatment strategy using the EUROPA-GEN or SMART-GEN treatment score yielded the highest net benefit at any treatment threshold considered. Hence, implementing these scores in clinical practice can improve the balance between number of patients treated and number of adverse cardiovascular outcomes prevented, irrespective of the treatment threshold.

Strengths of the present study include the large number of available events to derive treatment scores, the use of both clinical and genetic data and the use of existing and newly developed prediction scores. Further, the present study is the first to provide a treatment score to calculate an individualized estimate of the effect of perindopril. These estimates may help physicians to engage patients in shared-decision making by facilitating an appraisal of risks and benefits of treatment at an individual patient level. In addition,we evaluated the group level effects of implementing a prediction score in clinical practice,which is relevant to guideline makers. Potential limitations of our study also merit consideration. One of the main concerns of developing a new prediction score is that a score is likely to perform optimistically if tested in the sample from which it was derived [20]. To reduce optimism, we used penalized model estimation based on cross-validation and used a limited number of prespecified predictors. The effect-size of treatment interactions by genetic profile could not be based on external data, although the magnitude and directions of the interactions have been reproduced in ex-vivo experiments and in the PROGRESS trial [8,34]. In addition, the risk of chance findings was greatly reduced by only evaluating SNPs in 12 candidate genes that are part of biological pathways affected by ACE-i. This is different from genome wide association studies without a specific biological hypothesis. Other potential limitations include the generalizability of findings. As with the average trial result, the treatment prediction scores apply to patients who would be eligible for inclusion in the EUROPA trial. Since the number of female participants was relatively small, the models should be used with caution in female patients. Further, current predictions apply to a 5-year time period. In addition, the individualized effect estimates were not accompanied by uncertainty margins. In the setting of medical decision making, the interpretation of such margins can be difficult since the point estimate is the most likely value for an individual patient

[35]. Further, the use of a prediction score to select patients for treatment is more time consuming than treating everyone. However, the widespread use of electronic patient records in clinical practice may facilitate the use of prediction rules by automatically feeding information to risk calculators (Supplement Fig. 4). Nevertheless, genetic information regarding the SNPs that modify treatment effect is not routinely assessed in clinical practice. Given the promising results and potential clinical implications, external validation of treatment prediction algorithms including genetic information should be pursued.

In conclusion, treatment effect prediction scores based on clinical and genetic characteristics can quantify the ARR of major cardiovascular events for individual patients with sCAD. The use of a therapeutic prediction score in clinical practice can improve the balance between the number of patients treated and the number of events prevented compared with one-size-fits-all approaches such as treating no one or everyone.

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Conflicts of interest

W.R., M.B., R.F., K.F. and M.S. have received honoraria and research grants from Servier for the EUROPA-trial.

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All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.12.046.

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ONLINE SUPPLEMENT

Supplement Box 1. SMART treatment scores

Individual patient off-treatment risk: "treatment" = 0 (NO)
Individual patient on-treatment risk: "treatment" = 1 (YES)

A) SMART score

5-year MACE risk (%) = $(1 - 0.91^{\exp(A + 1.959)}) \times 100\%$

A = age * −0.0850 + age^2* 0.0015 + male sex * −0.1561 + SBP * 0.0043 + total cholesterol * 0.0959 + eGFR (MDRD) * −0.0532 + eGFR^2* 0.0003 + diabetes *0.2232 + current smoking * 0.2617 + prior CAD * 0.1401 + prior TIA or stroke 0.4058 + prior PVD * 0.2832 + years since first vascular event * 0.0229 + treatment * −0.2116

B) SMART-GEN score

5-year MACE risk (%) = $(1 - 0.91^{\exp(A + 2.164)}) \times 100\%$

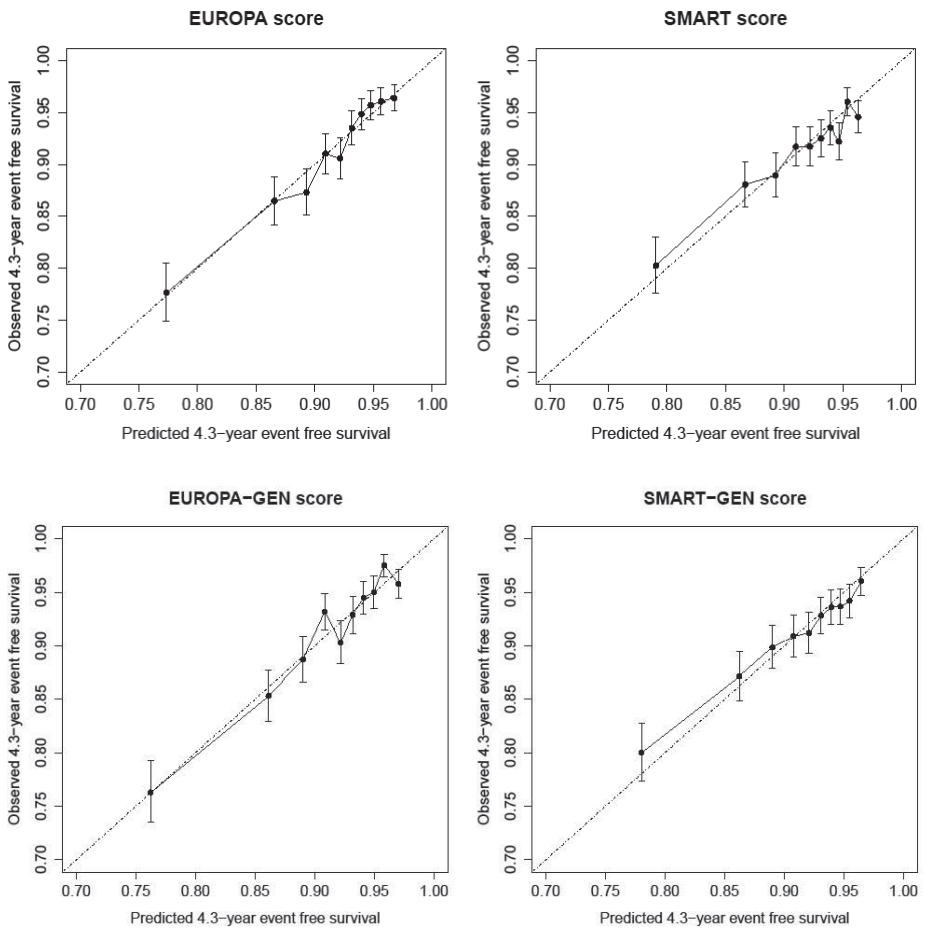
A = clinical SMART coefficients + genetic profile 1 * −0.1945 + genetic profile 2 * −0.4837 + treatment * −0.5159 + treatment & 2 unfavorable alleles * 0.2929 + treatment & ≥3 unfavorable alleles * 0.7231

Supplement table 1. Detailed model statistics

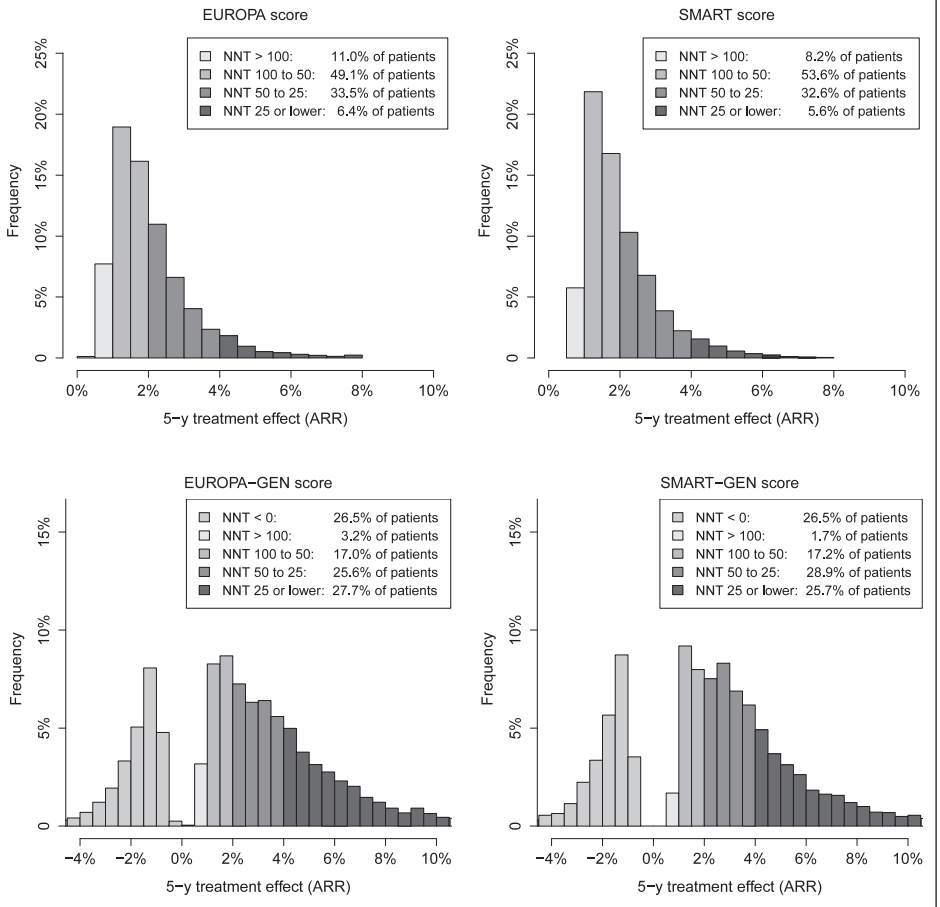
EUROPA score	*Coefficients ¶*	*HR*	*95% CI*	*LRT*	*p-value*
Age (years)*	−0.1324	1.37	1.22 - 1.52	54.2	<0.01
Age (years) squared	0.0013				
Female gender	−0.4643	0.63	0.52 - 0.76	26.6	<0.01
Systolic blood pressure (mm Hg)	0.0041	1.00	1.00 - 1.01	4.2	0.04
Total cholesterol (mmol/L)	0.1499	1.16	1.10 - 1.23	28.4	<0.01
eGFR (ml/min/1.73m^2)*	−0.0339	0.85	0.77 - 0.94	14.5	<0.01
eGFR (ml/min/1.73m^2) squared	0.0002				
BMI (kg/m^2)*	−0.2590	1.04	0.96 - 1.13	29.7	<0.01
BMI (kg/m^2) squared	0.0049				
Diabetes	0.4481	1.57	1.34 - 1.82	30.2	<0.01
Current smoking	0.3876	1.47	1.26 - 1.72	22.1	<0.01
Family history of CAD	0.1662	1.18	1.03 - 1.35	5.7	0.02
Prior MI	0.3671	1.44	1.26 - 1.66	28.6	<0.01
Prior TIA or stroke	0.4446	1.56	1.22 - 2.00	10.9	<0.01
Prior PVD	0.5092	1.66	1.39 - 1.99	27.8	<0.01
Prior coronary revascularization	−0.2235	0.80	0.71 - 0.91	12.4	<0.01
Symptomatic CAD†	0.3981	1.49	1.31 - 1.69	36.0	<0.01
Treatment	−0.2167	0.81	0.71 - 0.91	12.7	<0.01
EUROPA-GEN score[x]					
2 unfavorable allele	−0.2062	0.81	0.66 - 1.00	3.73	0.05
≥3 unfavorable alleles	−0.5112	0.60	0.47 - 0.77	17.63	<0.01
Treatment	−0.5466	0.58	0.46 - 0.72	24.77	<0.01
Treatment & 2 unfavorable allele	0.3207	1.38	0.99 - 1.93	3.65	0.06
Treatment & ≥3 unfavorable alleles	0.7498	2.12	1.49 - 3.04	17.31	<0.01

Supplement table 1. (*continued*)					
SMART-GEN score[x]					
2 unfavorable allele	−0.1945	0.82	0.66 - 1.01	3.37	0.07
≥3 unfavorable alleles	−0.4837	0.61	0.48 - 0.79	15.85	<0.01
Treatment	−0.5159	0.59	0.48 - 0.74	22.21	<0.01
Treatment & 2 unfavorable allele	0.2929	1.35	0.97 - 1.88	3.11	0.08
Treatment & ≥3 unfavorable alleles	0.7231	2.07	1.45 - 2.96	16.23	<0.01

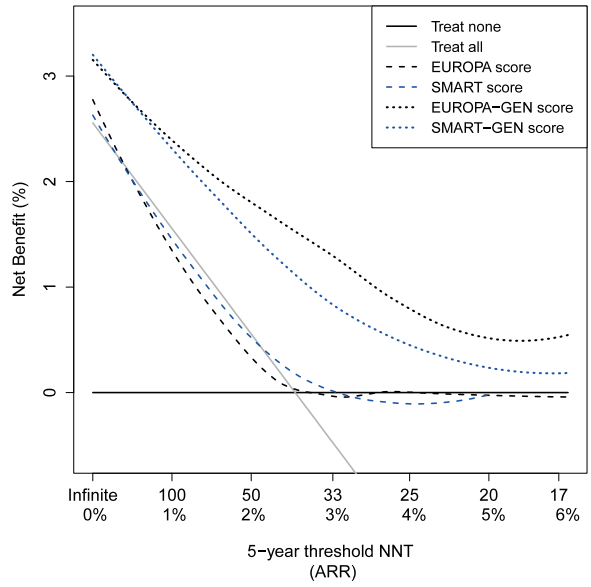
eGFR: estimated glomerular filtration rate (CKD-EPI equation), LRT: likelihood ratio test. † Agina pectori or previous heart failure. ¶ Coefficients were shrunken to increase external validity, whereas unbiased HRs and statistics were derived from the unpenalized Cox models. *Risk model contains a linear and squared term for age, BMI and eGFR. Therefore the LRT and p-value for age, BMI and eGFR is computed for both terms together. Futhermore HR were computed for the difference between the study population's 75th and 25th percentile of age (67 vs 53), BMI (29.4 vs 25.0) and eGFR (87 vs 64). [x]The additional variables to the EUROPA and SMART risk score are presented here. Estimates of the clinical predictors in the EUROPA-GEN score were comparable to the estimates of the EUROPA score.



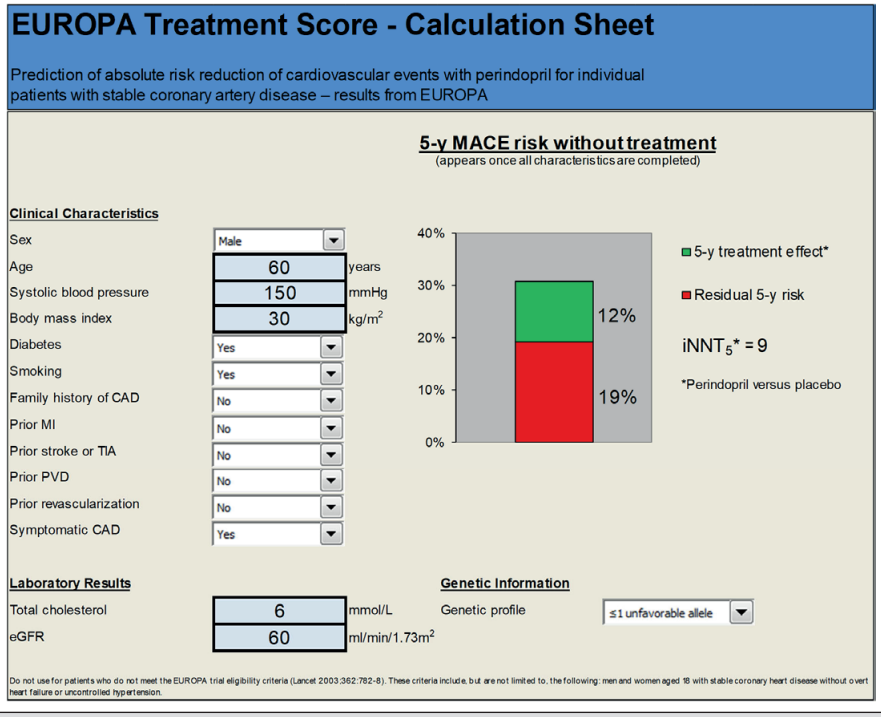
Supplement figure 1. Calibration plots of predicted versus observed 4.3-year absolute risk of major cardiovascular events (MACE) in deciles.



Supplement figure 2. Distribution of 5-year absolute treamtent effect of major cardiocvascular events (MACE) with perindopril for individual patients with sCAD based on EUROPA and SMART scores.



Supplement figure 3. Net benefit curves of different treatment strategies for major cardiovascular events (MACE).



Supplement figure 4. Calculation sheet

CORONARY VULNERABILITY

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INDIVIDUALIZED ACE-INHIBITOR THERAPY IN STABLE CORONARY ARTERY DISEASE BASED ON CLINICAL AND PHARMACOGENETIC DETERMINANTS; THE PERGENE RISK MODEL

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ABSTRACT

Background: Patients with stable CAD constitute a heterogeneous group in which the treatment benefits by ACE-inhibitor therapy vary between individuals. Our objective was to integrate clinical and pharmacogenetic determinants in an ultimate combined risk prediction model.

Methods and results: Clinical, genetic and outcomes data were used from 8726 stable CAD patients participating in the EUROPA/PERGENE trial of perindopril versus placebo. Multivariable analysis of phenotype data resulted in a clinical risk score (range: 0-21 points). Three SNPs (rs275651 and rs5182 in the angiotensin-II type I-receptor gene and rs12050217 in the bradykinin type I-receptor gene) were used to construct a pharmacogenetic risk score (PGXscore, range: 0-6 points). 785 patients (9.0%) experienced the primary endpoint of cardiovascular mortality, non-fatal MI or resuscitated cardiac arrest during 4.2 years of follow-up. Absolute risk reductions ranged from 1.2% to 7.5% in the 73.5% of patients with PGXscore of 0-2. As a consequence, estimated annual numbers needed to treat ranged from as low as 29 (clinical risk score ≥10 and PGXscore of 0) to 521 (clinical risk score ≤6 and PGXscore of 2). Furthermore, our data suggest that long-term perindopril prescription in patients with a PGXscore of 0-2 is cost-effective.

Conclusions: Both baseline clinical phenotype, as well as genotype determine the efficacy of widely prescribed ACE-inhibition in stable CAD. Integration of clinical and pharmacogenetic determinants in a combined risk prediction model demonstrated a very wide range of gradients of absolute treatment benefit.

The European Trial on Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA) and the Heart Outcomes Prevention Evaluation (HOPE) have demonstrated the effectiveness of angiotensin-converting enzyme (ACE)-inhibitors perindopril and ramipril respectively, by reduction of mortality and morbidity from cardiovascular events among patients with stable coronary artery disease (CAD)[1,2]. Consequently, ACE-inhibitors are recommended in clinical guidelines on secondary prevention in patients with stable CAD and hence widely used in this population.[3–5]

However, patients with stable CAD constitute a heterogeneous group in which the absolute risk of cardiovascular complications varies between individuals.[6,7]

Several approaches towards the identification of those patients that are most likely to benefit from ACE-inhibitor therapy have previously been reported. A previously published post-hoc analysis of the EUROPA trial studied baseline clinical risk factors such as age, gender, smoking, cholesterol and blood pressure levels.[6] A risk score founded on such baseline clinical risk factors was able to identify patients at high, medium and relatively low absolute risk (>3%, 1-3% and 1% per annum respectively) of experiencing cardiovascular death, non-fatal myocardial infarction (MI) and resuscitated cardiac arrest.[6] In contrast to the *absolute* treatment benefit, the *relative* treatment effect of perindopril, however, was not modified by the baseline level of risk.[6] Similar conclusions were drawn after investigation of the relation between treatment benefit by perindopril and baseline renal function or the degree of blood pressure reduction.[8–10]

A novel approach towards selection of those that are likely to respond (or not) to ACE-inhibitor therapy is to identify information on genetic variation among patients.[11] A recent publication by our group demonstrated that genetic variation in the renin-angiotensin-aldosterone system (RAAS) and the kallikrein-bradykinin (KB) pathway is associated with the treatment benefit of perindopril.[12] Three single nucleotide polymorphisms (SNPs), two of which in the angiotensin-II type I (AT1) receptor gene and one in the bradykinin type I (BK1) receptor gene, were used to construct an integer-based pharmacogenetic risk score (PGXscore), ranging from 0 to 6 points.[12] We were able to identify two distinct subgroups within the overall study population of 8726 patients on the basis of this PGXscore.[12] One subgroup (73.5% of the patients) was characterized by a more pronounced treatment benefit, whereas no treatment benefit was apparent in the remaining 26.5% of patients.

This current analysis is an ultimate extension of both the previously published clinical risk model[6] and pharmacogenetic risk profile.[12] Its purpose is two-fold: 1) to investigate the relation between identified genetic determinants of treatment benefit and different levels of baseline clinical risk; 2) to integrate clinical and pharmacogenetic determinants in an ultimate combined risk prediction model.

METHODS

Study population and design

The PERindopril GENEtic association study (PERGENE) is a substudy of the EUROPA trial. The designs of both studies have been reported previously.[1,12] In brief, the EUROPA trial was a randomized, double-blinded, placebo-controlled study designed to assess the effect of perindopril (8 mg daily) on the combined primary endpoint of cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest in 12218 patients with stable CAD, but without overt heart failure or uncontrolled hypertension. The use of perindopril resulted in a 20% relative risk reduction (adjusted HR 0.80, 95% CI: 0.71-0.91) in the rate of the primary endpoint during a mean follow-up of 4.2 years.[1]

A DNA bio-bank was established within the EUROPA trial for the purpose of the PERGENE substudy, which investigates whether genetic variation is a determinant of the risk of future adverse cardiovascular outcome and/or treatment benefit by the use of perindopril.[11] DNA was successfully isolated in 9454 patients, using an automated isolation process.[11] Comprehensive coverage of genetic variation in both the RAAS and KB pathways was ensured by a haplotype-tagging-single nucleotide polymorphism (ht-SNP) procedure in 12 candidate genes, as described in detail previously.[11,12]

Our study was approved by the Institutional Review Board of every participating center and written informed consent for genetic association analyses was obtained from all patients.

Clinical risk score

Univariable and multivariable Cox' proportional hazard regression analyses were performed to study the relation between the primary endpoint (cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest) and baseline clinical patient characteristics, such as demographic and clinical variables, medical history, laboratory tests and concomitant medication. Interaction by treatment was investigated for each clinical characteristic. A final multivariable clinical risk model was constructed using a backward stepwise elimination procedure in which removal testing was based on the probability of the likelihood-ratio statistic based on the maximum partial likelihood estimates. In order to develop a clinical risk-scoring system, the log HRs from the final multivariable model were converted to an estimated risk score.[6,13] Clinical risk scores were calculated for each of the patients of the currently described population (only those trial participants of whom both baseline clinical characteristics and (pharmaco)genetic profile were complete). The study population was divided into tertiles in order to distinguish low, medium and high clinical risk profiles.

Pharmacogenetic risk profile and replication

The PERGENE substudy assessed 52 SNPs with the use of Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) and Sequenom (San Diego, CA, USA) mass-spectrometric genotyping. Quality control for the accuracy of genotyping involved testing duplicates from a randomly selected group of samples (5%) for concordance between samples (always >99% replication). Individual SNP call rates ranged between 95 and 98%. To ensure DNA quality, only patients who were successfully genotyped for more than 90% of the selected 52 SNPs were included in the PERGENE analyses (n = 8907).[12]

Seven SNPs have previously been reported to significantly modify the treatment effect of perindopril in univariate analyses.[12] After multivariate adjustment and correction for multiple testing, three SNPs remained significant modifiers of the perindopril treatment effect: rs275651 and rs5182 in the angiotensin-II type I (AT1) receptor gene and rs12050217 in the bradykinin type I (BK1) receptor gene. These three SNPs formed the foundation of a previously published PGXscore, ranging from 0 to 6 points, which was constructed by counting the number of alleles that were associated with a decreased benefit of perindopril treatment.[12] The association between the PGXscore and treatment benefit by perindopril, as found in PERGENE,[12] was replicated in the PROGRESS study, which investigated the treatment effect of perindopril in patients with cerebrovascular disease.[14]

Statistical analysis

Differences in baseline clinical characteristics between low, medium and high clinical risk groups were assessed by chi-square tests in case of categorical data or one-way analysis of variance in case of continuous data. A multivariate Cox proportional hazards regression model was fitted with the following covariates: clinical risk score, PGXscore, treatment and treatment*PGXscore interaction (full model). The baseline hazard function $H_0(t)$ was estimated by dividing the cumulative hazard at the end of follow-up through the exponential function of the mean of the covariates. The cumulative survival under perindopril treatment versus placebo at the median follow-up of 4.2 years was calculated for each clinical risk score within the separate pharmacogenetic risk strata as follows: S (4.2years) = 0.033975 * exp(0.196*clinical risk score – 0.203*PGXscore – 0.793*treatment – 0.318*interaction term) With respect to "treatment" placebo was defined as 0 and perindopril treatment as 1. The "interaction term" was the multiplication of the PGXscore * treatment. Absolute and relative risks, as well as crude and adjusted hazard ratios (HR) are presented with 95% confidence intervals (CI). Numbers needed to treat (NNT) in order to prevent one event per annum were calculated as the inverse of the absolute risk reduction at the mean clinical risk scores per stratum.

The performance of the model consisting of clinical risk score only was compared by two different methods with the full model with respect to discrimination. First, the c-index and areas under the two receiver operating characteristic curves were compared by a nonparametric method, as previously described by de Long et al.[15] Secondly, the difference in model-based discrimination slopes was evaluated through integrated discrimination improvement (IDI).[16] Calibration of both the model consisting of clinical risk score only and the full model was tested with the Hosmer-Lemeshow [H-L] goodness-of-fit test. All statistical tests were two-sided with a type I error level of 0.05, except for the IDI for which a conservative significance level of 0.01 was maintainted.[16]

We performed a cross-validation within our own dataset by bootstrap methods as suggested by Harrell et al.[17] We constructed 300 bootstrap samples (training) from the full original sample with the same size as the original (test). Models were built in the training sets. C-indices were then obtained in these training sets ($C_{training}$) and compared with the c-indices of the models when applied to the test set (C_{test}). The optimism in the fit from bootstrap sample i is defined as $O_i = C_{i,training} - C_{i,test}$. We report the mean O of these optimism estimates. Analyses were performed with IBM SPSS statistics version 23.0 and STATA version 12.

Cost-effectiveness analysis

We examined the potential cost-effectiveness of the combined clinical risk score and PGXscore. The time horizon was restricted to the duration of the EUROPA trial/PERGENE study (mean follow-up of 4.2 years). Costs were set at 15 euros for the analysis of the three SNPs of the PGXscore, 50 euros for perindopril (based on the current price of perindopril 8 mg tablets in the Netherlands), and 3,000 euros for a clinical event (a weighted average of the costs of treating myocardial infarction and the costs of cardiac death). The health loss of a clinical event within the trial duration was set at 0.6 years (a weighted average of the relative frequency and life-years lost from myocardial infarction (0 years) and cardiac death (2 years).

The following patient management strategies were examined, against the strategy of no perindopril treatment (as the comparator):

1) Pharmacogenetic testing only in patients with a high clinical risk score and perindopril treatment only if PGXscore=0-2
2) Pharmacogenetic testing only in patients with a medium or high clinical risk score and perindopril treatment only if PGXscore=0-2
3) Pharmacogenetic testing in all patients and perindopril treatment only if PGXscore=0-2
4) Perindopril treatment in all patients irrespective of PGX score.

RESULTS

Complete data on baseline clinical patient characteristics and (pharmaco)genetic profile were obtained for 8726 patients (of which 4338 were allocated to perindopril and 4388 to placebo). Median follow-up was 4.2 years (interquartile range 4.0-4.5 years), during which 785 patients (9.0%) experienced the primary endpoint of cardiovascular mortality, non-fatal MI or resuscitated cardiac arrest. Treatment with perindopril was protective in the overall study population; the number of patients on perindopril treatment that experienced the primary endpoint was 346 (8.0%) versus 439 (10.0%) on placebo (adjusted HR 0.80, 95% CI: 0.68-0.92) Baseline characteristics of the overall study population and various subgroups according to the clinical risk level are provided in table 1. Interaction between study treatment and clinical characteristics (including concomitant medication) was not found.

Table 1. Baseline study population characteristics

	Total population	CLINICAL RISK LEVEL			p-value *
		Low	Medium	High	
N (%)	8726	3167 (36.3)	3474 (39.8)	2085 (23.9)	
Age, years	59.8 (9.3)	57.7 (8.0)	59.4 (9.2)	63.8 (10.0)	<0.001
Male gender (%)	85.5	81.5	87.6	88.2	<0.001
Hypertension (%) †	29.0	23.0	28.0	39.0	<0.001
Diabetes mellitus (%)	13.0	4.0	11.0	30.0	<0.001
Hypercholesterolemia (%) ‡	63.0	69.0	60.0	58.0	<0.001
Current smoking (%) §	15.0	6.0	16.0	25.0	<0.001
Obesity (BMI>30 kg/m^2) (%)	21.3	8.2	24.0	36.7	<0.001
Symptomatic CAD (%) ||	25.4	9.5	25.9	48.9	<0.001
Family history of CAD (%)	27.0	22.0	29.0	32.0	<0.001
Previous MI (%)	65.0	44.0	73.0	84.0	<0.001
Previous revascularisation (%)	55.0	75.0	49.0	33.0	<0.001
Previous stroke or PAD (%)	8.9	0.8	5.2	27.5	<0.001
CONCOMMITANT MEDICATION					
Platelet-inhibitors (%)	92.0	94.0	92.0	89.0	<0.001
Beta-blockers (%)	63.0	62.0	65.0	63.0	0.104
Lipid-lowering agents (%)	55.0	64.0	53.0	46.0	<0.001
Calcium-antagonists (%)	32.0	29.0	31.0	37.0	<0.001
Systolic blood pressure (mmHg)	136.9 (15.2)	132.7 (13.9)	137.7 (15.1)	142.1 (15.5)	<0.001
Diastolic blood pressure (mmHg)	81.8 (8.1)	80.6 (7.9)	82.4 (8.1)	82.7 (8.3)	<0.001
Creatinine clearance (µmol/L) #	86.5 (25.7)	88.9 (22.2)	87.7 (26.6)	80.9 (28.3)	<0.001
Total cholesterol (mmol/L)	5.4 (1.0)	5.1 (0.9)	5.5 (1.0)	5.7 (1.1)	<0.001

Table 1. *(continued)*

	Total population	CLINICAL RISK LEVEL			p-value *
		Low	Medium	High	
OUTCOME					
Randomization, allocation to perindopril (%)	49.7	51.0	48.9	49.5	0.296
Primary endpoint	**9.0**	**4.6**	**8.8**	**16.2**	**<0.001**
Systolic / diastolic blood pressure reduction by perindopril (mmHg)**	8.6 / 4.0	7.3 / 3.9	9.2 / 4.1	9.6 / 4.1	<0.001 / 0.416
RISK SCORE		**0-6**	**7-9**	**10-21**	
Mean clinical risk score	**7.67 (2.83)**	**4.84 (1.20)**	**7.93 (0.80)**	**11.53 (1.74)**	**N.A.**
Mean pharmacogenetic risk score	**1.82 (1.13)**	**1.82 (1.12)**	**1.82 (1.12)**	**1.86 (1.11)**	**0.435**

Summary statistics for continuous variables are presented as mean (standard deviation). Categorical data are summarized as percentages. CAD=coronary artery disease MI=myocardial infarction N.A.=not applicable PAD=peripheral artery disease

* For differences between low, medium and high clinical risk levels.

† Blood pressure >140/95 mm Hg or receiving antihypertensive treatment.

‡ Previously known total cholesterol >6·5 mmol/L or receiving lipid-lowering treatment.

§ Use of tobacco within the last month

|| Stable angina pectoris or history of congestive heart failure

Estimation by Cockroft-Gault equation

** Blood pressure reduction was calculated as the mean difference in blood pressure from screening visit 1 to randomization after the 4-week run-in period of the Europa-trial in which all patients were treated with perindopril.

Significant baseline clinical risk predictors and the point-scoring system, which derived from backward elimination, are presented in table 2. The log HRs from the final multivariable model that were converted to the clinical risk score are provided in the Online data supplement. The clinical risk score could theoretically range from 0-32, yet calculated individual scores within our study population ranged from 0-21 with a mean value of 7.67 +/- 2.83 (figure 1). The 33rd and 67th percentiles were at 6.00 and 9.00 points respectively, and used as cut-offs in order to distinguish low, medium and high clinical risk levels. The skewness of the distribution (figure 1) prevented formation of three groups of similar size. It should be noted that the high risk group consists of 23.9% of the overall study population (tables 1 and 3). Incidences of all known baseline cardiovascular risk factors were highest in the higher clinical risk groups (table 1), with exception of previously diagnosed hypercholesterolemia, which actually was lowest in the high-risk subgroup. In accordance, high-risk patients also presented with the lowest rate of statin use. These findings, however, were counterbalanced by the fact that patients in the high-risk subgroup did have the highest total cholesterol levels (table 1).

The primary endpoint rates in the low, medium and high clinical risk groups were 4.6%, 8.8% and 16.2% respectively (p<0.001, table 1). These differences in event rate

Table 2. Clinical risk scores of baseline risk parameters

Continuous clinical risk parameters				**Clinical Risk Score points**
Age	Systolic blood pressure	Creatinine clearance	Total cholesterol mmol/L (mg/dL)	
<67	≤130	>70	≤3.5 (≤135)	**0**
67-69	>130-≤160	>55-≤70	>3.5-≤5.0 (>135-≤193)	**1**
70-72	>160	>35-≤55	>5.0-≤6.5 (>193-≤251)	**2**
73-76		≤35	>6.5-≤8.0 (>251-≤309)	**3**
77-79			>8.0 (>309)	**4**
80-82				**5**
83-85				**6**
>85				**7**
Dichotomous clinical risk parameters				
Previous stroke or PAD				**3**
Male gender				**2**
Obesity (BMI>30 kg/m^2)				**2**
Current smoking				**2**
Symptomatic CAD				**2**
Diabetes mellitus				**2**
Previous MI				**2**
Family history of CAD				**1**
Previous revascularisation				**–1**

The range of clinical risk scores = 0-32 and points for each of applicable variables need to be added to each other. CAD=coronary artery disease MI=myocardial infarction PAD= peripheral artery disease.

Table 3 . Distribution of patients over clinical and pharmacogenetic risk strata

Clinical risk level	**Low**	**Medium**	**High**
Clinical risk score	**0-6**	**7-9**	**10-21**
Pharmacogenetic risk score N(%)			
0	362 (4.1)	390 (4.5)	232 (2.7)
1	945 (10.8)	1037 (11.9)	618 (7.1)
2	1027 (11.8)	1144 (13.1)	655 (7.5)
≥3	833 (9.5)	903 (10.3)	580 (6.6)

Treatment benefit of perindopril was only demonstrated within the group of patients with pharmacogenetic risk scores < 3 (N=6410, 73.5% of the total study population). The Linear-by-Linear Association p-value for the entire table is 0.43.

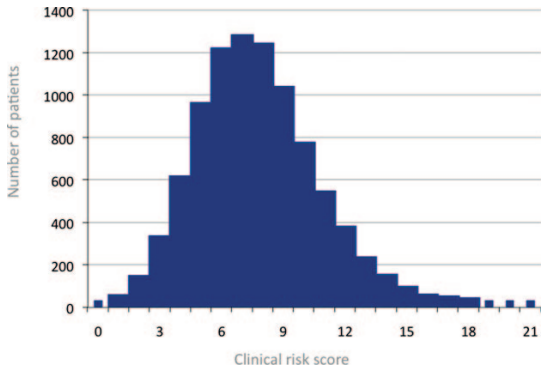

Figure 1. Clinical Risk Score distribution
The mean value of the clinical risk score (N=8726) was of 7.67 +/- 2.83

can be explained by the observed differences in baseline clinical risk factors, but not by confounding due to study drug allocation, since the latter was similar over the three clinical risk strata (p=0.296, table 1).

Adjusted HRs for the treatment effect of perindopril were 0.72, 0.70 and 0.91 for the lowest to highest clinical risk tertiles respectively. Heterogeneity of treatment effect was tested and ruled out (p=0.31). Thus, the relative treatment benefit was not modified by the baseline clinical risk level. However, baseline clinical risk level did modify *absolute* risk reductions. The use of perindopril in the overall study population (n=8726) resulted in a 2.23% risk reduction of the primary endpoint (95% CI: 1.03 – 3.44, annual NNT 189, 95% CI:122-401). However, absolute risk reductions varied from 1.24% to 2.17% and 3.97% in the lowest, medium and highest clinical risk tertiles. As a consequence, NNTs were inversely related to increasing clinical risk scores (table 4).

Table 4. Numbers needed to treat (per annum)

Clinical risk level	**Low**	**Medium**	**High**
Clinical risk score	**0-6**	**7-9**	**10-21**
NNT per clinical risk stratum	382	218	119
NNT per pharmacogenetic risk stratum			
0	93	54	29
1	164	92	50
2	521	298	164
≥3*	*–529*	*–302*	*–164*

*Stratum with non-significant risk increase due to use of perindopril.

The pharmacogenetic risk scoring system, based on the previously identified 3 SNPs, is presented the Online data supplement. Risk alleles for lack of treatment benefit were T, C, and G for Rs275651, Rs5182 and Rs12050217 respectively. The individual PGXscores range from 0-6 points with a mean value of 1.82 +/- 1.13 (table 1). Significant heterogeneity of treatment effect across pharmacogenetic profiles was observed. A pronounced treatment benefit was observed in 6410 patients (73.5%) with PGXscore <3 (adjusted HR 0.67, 95% CI: 0.56-0.79), whereas no benefit was observed in the remaining subgroup of 2316 patients (26.5%) with PGXscore ≥3 (adjusted HR 1.26, 95% CI: 0.97-1.67) (table 3 for patient distribution).

The use of perindopril in patients with PGXscores of 0 and 1 point resulted in absolute risk reductions of 7.50% (95% CI: 3.69 – 11.73) and 4.30% (95% CI: 2.00 – 6.53) respectively. Consequently, annual numbers needed to treat were 55 (95% CI: 113 – 38) for patients with a PGXscore of 0 and 97 (95% CI: 210 – 63) for patients with a PGXscore of 1. The point estimate of the absolute risk reduction associated with the use of perindopril in the subgroup of PGXscore of 2 was in the same positive direction, yet non-significant (1.34%, 95% CI: −0.77 to 3.47 and NNT (per annum)= 311, 95% CI: −546 to 122).

In contrast, a non-significant estimated absolute risk increase of 1.32% was observed in patients with a PGXscore ≥3 using perindopril (95% CI for risk increase −0.97 to 3.67 and NNT (per annum)= −315, 95% CI: −118 to 433).

Combined baseline clinical and pharmacogenetic risk profiles

Mean pharmacogenetic risk scores were identical over all three clinical risk strata (p=0.435, table 1) and formal testing did not trace interaction between the clinical and PGXscores. The distribution of patients over the various clinical and pharmacogenetic risk strata is given in table 3. Figures 2a-2d describe the relation between absolute risks of the primary endpoint, clinical risk profile and treatment for each of the separate pharmacogenetic risk strata. Lack of treatment benefit was observed across the entire spectrum of clinical risk in patients with a PGXscore ≥3 (figure 2d and figure 3).

Increasing clinical risk scores led to increasingly pronounced risk differences between perindopril and placebo in all pharmacogenetic strata. Hence, extremes of treatment effect were found in patients with high clinical risk profiles. For example, the use of perindopril in patients with a clinical risk score of 19 resulted in an estimated absolute risk reduction of 28.42% (95% CI: 22.46 – 34.09) in case of a PGXscore of 0 versus an estimated risk increase of 5.82% (95% CI: 1.78 – 9.83) in case of a PGXscore ≥3 (figure 3). Concordantly, NNTs decreased in subgroups with higher clinical risk profiles and lower PGXscores, both of which were associated with more pronounced treatment effects (table 4). Estimated numbers needed to treat were as low as 29 (95% CI: 17 – 113) in patients with a high clinical risk profile and a PGXscore of 0, whereas those with a low

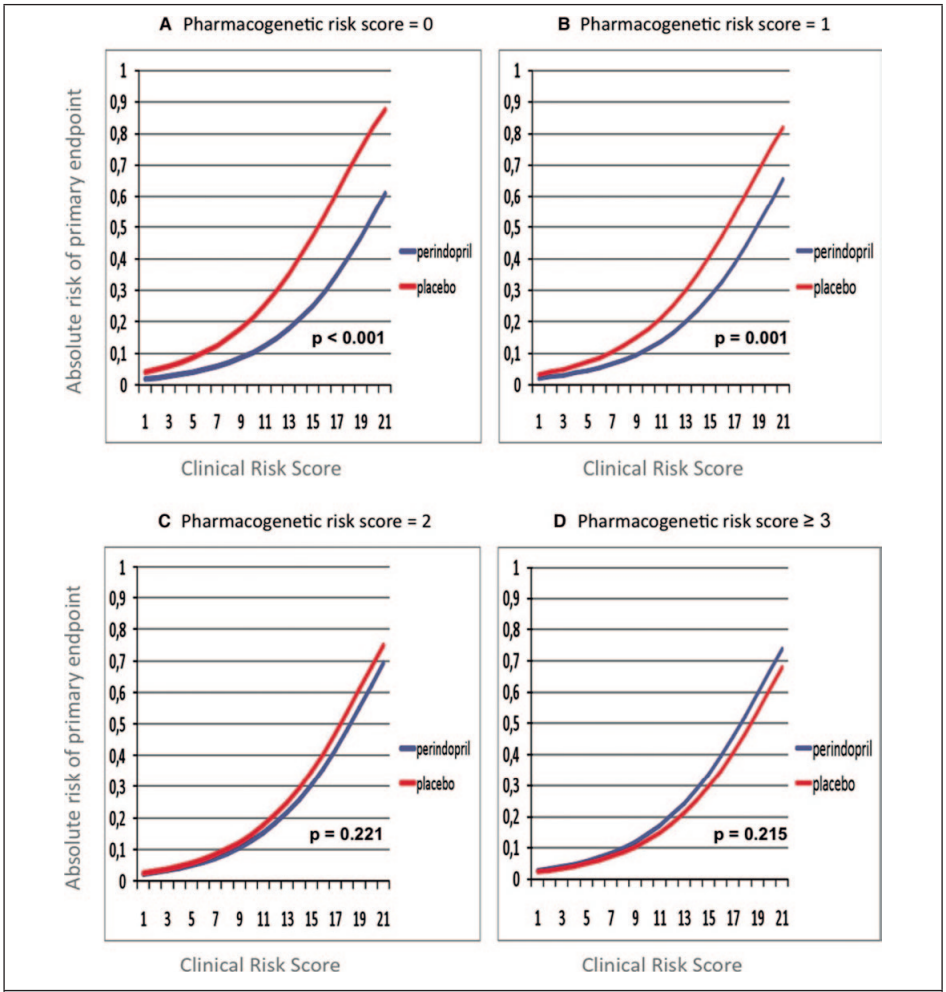

Figure 2. Absolute risks of the primary endpoint across different clinical and pharmacogenetic risk strata

P-values were derived from multivariate Cox proportional hazards regression models fitted with the following covariates: clinical risk score, PGXscore, treatment and treatment*PGXscore interaction. PGX-score= pharmacogenetic risk score

clinical risk profile and a PGXscore of ≥3 did not experience any benefit (NNT= −529, 95% CI: −105 to 189).

In separate analyses with only cardiovascular mortality or non-fatal MI as sole endpoint, we observed directional concordance, compared to the presented analysis of the combined primary endpoint, with respect to NNTs over the various clinical and pharmacogenetic risk strata.

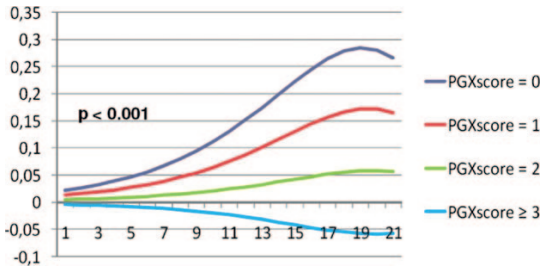

Figure 3. Absolute risk reduction (Y-axis on a 0 to 1 scale) by perindopril across different levels of clinical (X-Axis) and pharmacogenetic risk
PGXscore = pharmacogenetic risk score

Discrimination and calibration of the clinical and combined risk models

Calibration and discrimination were assessed for two models: A) the model consisting of clinical risk score only, and B) the full model consisting of clinical risk score, PGXscore and treatment*PGXscore interaction.

Addition of pharmacogenetic information on top of clinical risk profile resulted in better discrimination. The c-index for the full model (0.68, 95% CI: 0.66-0.70) was significantly higher than the c-index for the model consisting of the clinical risk score only (0.66, 95% CI: 0.64-0.68) (p=0.0015). The full model also resulted in a significantly better discrimination when assessed with integrated discrimination improvement (magnitude of increase in IDI: 0.00472, p=0.0002). Validation of both models by bootstrap methods showed that the bias in the estimated discrimination performance (c-index) is likely to be small, since the mean optimism estimates were only 0.006 and 0.007 for the clinical

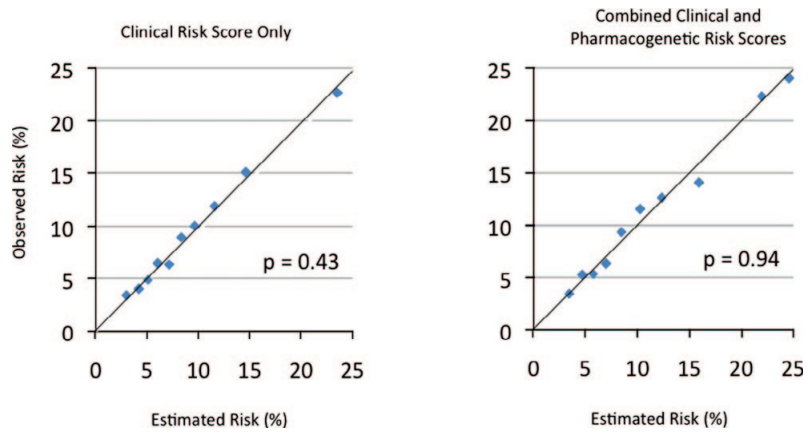

Figure 4. Observed versus estimated risks according to the clinical and combined (full) risk prediction models
P-values were derived from Hosmer-Lemeshow goodness-of-fit tests

risk score and the full model, respectively. Finally, The H-L goodness-of-fit tests were non-significant (p=0.43 for the model with the clinical risk score only and p=0.94 for the full model), indicating adequate calibration for both models (figure 4).

Cost-effectiveness of tailored perindopril treatment on the basis of pharmacogenetic testing

The results of the cost-effectiveness analysis, against the strategy of no treatment with perindopril as comparator, are displayed in table 5.

The highest number of gained life-years is observed in strategies 3 and 4. Strategy 3 implies that all patients are genetically tested and only those with a PGXscore of 0-2 are treated with perindopril. Strategy 4 implies that none of the patients are genetically tested and all are treated with perindopril. Strategy 4 however is dominated by strategy 3. The lower incremental cost-effectiveness ratio (ICER) of strategy 3 indicates that tailored perindopril therapy on the basis of the PGXscore will ultimately reduce costs, with a similar effectiveness in terms of gained life-years.

Strategy one results in the least life-years gained, but also in the least costs and the lowest ICER and therefore may be an option when strictly reasoning from the cost perspective alone.

Table 5. Costs, gained life-years and incremental cost-effectiveness ratio of various treatment strategies, against the strategy of no treatment with perindopril as comparator.

Strategy	Number of patients treated with perindopril, N (%)	Incremental costs (weighted)	Life-years gained (weighted)	ICER
1. Pharmacogenetic testing only in patients with a **high clinical risk score** (≥10) and perindopril treatment only if **PGXscore=0-2**	1505 / 8726 (17.2)	30.38	0.0017	18,139
2. Pharmacogenetic testing only in patients with a **medium or high clinical risk score** (≥7) and perindopril treatment only if **PGXscore=0-2**	4076 / 8726 (46.7)	90	0.0032	27,987
3. Pharmacogenetic testing in **all patients** and perindopril treatment only if **PGXscore=0-2**	6410 / 8726 (73.5)	147	0.0040	36,743
4. Perindopril treatment in **all patients irrespective of PGXscore**	8726 (100)	232	0.0035	67,230

The time horizon was restricted to the duration of the EUROPA trial/PERGENE study (mean follow-up of 4.2 years). Costs are in euros. PGXscore = pharmacogenetic risk score. ICER = incremental cost-effectiveness ratio

DISCUSSION

The present study highlights that clinical as well as pharmacogenetic determinants independently modify *absolute* treatment benefit by ACE-inhibitor perindopril in a population of patients with stable CAD. Moreover, both clinical and pharmacogenetic profiles could be expressed in risk scores that are fairly simple to use for clinical decision-making. We propose the use of a PGXscore on top of known clinical risk factors for better risk stratification and more concrete estimation of absolute treatment benefits of ACE-inhibitor therapy in daily clinical practise. Increasing clinical risk scores and decreasing PGXscores were consistently and positively related to the absolute treatment benefit by ACE-inhibitor perindopril. Impressive risk gradients and, as a consequence, important differences in NNTs were found across various subgroups. The annual NNT in the overall study population was 189, whereas estimates as low as 106 for the entire clinical high-risk subgroup and even 29, in case of a combined high clinical risk profile and a PGXscore of 0, were observed. On the other hand, the entire subgroup of patients with a PGXscore ≥3 (26.5% of the overall study cohort) was characterized by a lack of treatment benefit, which was consistent across all three clinical risk levels.

The clinical risk score in our study was based upon easily obtainable traditional risk factors that have repeatedly proven to be valuable predictors.[18–20] The full model predicted the highest *absolute* risk reductions in patients with higher clinical risk profiles. In this regard, it remains important to emphasize that formally no heterogeneity of relative treatment effect was found across the various clinical risk levels. Furthermore, the mean clinical risk score in the high-risk level was 11.53. Scores of e.g. 19 can therefore be regarded as extremely high. Such extreme risk scores were underrepresented in our RCT data, but nevertheless such patients do present themselves in clinical practise. It is plausible that in such extremely high-risk individuals, the risk is largely determined by the aforementioned risk factors, and that an ACE-inhibitor alone will have *relatively* less effect on survival. In other words, the magnitude of both controllable and uncontrollable clinical risk factors in such a patient could have a *relatively* more profound effect on the risk of reaching the primary endpoint, than the potential *relative* treatment benefit by an ACE-inhibitor alone. Obviously, the absolute risk benefit will remain high in such patients and treatment with an ACE-inhibitor should therefore be warranted. This finding, however, once again emphasises the necessity of proper management of all controllable risk factors in patients with stable CAD.

With this in mind, it is remarkable that a history of coronary revascularization was associated with a modestly reduced risk for the primary endpoint (−1 point) in the presented risk model. This particular observation should be interpreted with some reservation, since several specifically designed trials, such as RITA-2,[21] COURAGE[22] and BARI 2D[23] failed to demonstrate survival benefit of coronary revascularisation over optimal medical therapy.

The pharmacogenetic risk score in our study was based upon three SNPs that have previously emerged after comprehensive coverage of the RAAS and KB systems and subsequent correction for multiple testing.[12] Furthermore, the pharmacogentic risk score has previously been replicated in participants of the PROGRESS-trial.[12] Clinical risk factors,[6] renal function,[8] degree of blood pressure reduction[9] and a number of biomarkers[24,25] have been explored within the EUROPA trial, yet only pharmacogenetic information has permitted to distinguish responders to perindopril from non-responders (26.5% of all patients). Furthermore, the PGXscore accentuated striking differences in absolute treatment benefits of ACE-inhibitor therapy within each of the separate clinical risk strata.

The data that are presented here are unique. Although it is widely recognized that both phenotype as well as genotype play a fundamental role in health and disease outcome, very few reports exist that actually combine both for prognostication. To our best knowledge, this is the first and only manuscript that combines clinical and genetic information in patients with CAD. Pharmacogenetic information is successfully translated into a potential clinical utility to study the gradients of treatment effect by an ACE-inhibitor. The sample size is large and various additional qualities of a well-designed placebo-controlled double-blinded RCT, such as high quality phenotypical data and independent event adjudication are apparent. Previous studies that have investigated the relation between genetic variation and treatment benefit by ACE-inhibitor therapy usually were characterized by small sample sizes and non-randomized designs without placebo controls.[26] Only two studies with large sample sizes have been reported. Harrap and colleagues studied macrovascular events, dementia and cognitive decline in 5688 patients with a history of cerebrovascular disease in the PROGRESS study and found no interaction between genetic variation and treatment benefit by perindopril.[27]

Negative findings were also published by the GenHAT investigators, who studied cardiovascular mortality and non-fatal MI in 7528 patients on a lisinopril based regimen in the setting of an active-controlled RCT.[28] These two studies obviously differ from our present study in the type of study population, endpoints and study drug. The most remarkable difference with our study, however, is the fact that both studies solely focussed on a single ACE insertion/deletion polymorphism, thus not taking account of the full complexity of the RAAS and KB systems. Furthermore, our PGXscore was replicated in the PROGRESS study, in which a similar direction and magnitude of pharmacogenetic interaction was observed.[12]

Our findings also have some limitations. This study describes differences in treatment benefit across a range of clinical and genetic subgroups. The constituents of the clinical scoring system are all well established cardiovascular risk factors and the PGXscore has been replicated. Still, it is important to realize that, in general, any post-hoc analysis based on subgroups should primarily be regarded as hypothesis-generating. Confirmation of

our findings in other large datasets would invigorate the presented conclusions and derived clinical implications. The EUROPA trial was powered for detection of treatment benefit for the entire study population irrespective of clinical or pharmacogenetic risk categories. Thus lack of power cannot be excluded as an explanation for the observed non-significant treatment benefit in the higher PGX scores. On the other hand it must be noted the absolute numbers of study participants in PGXscores ≥ 2 are higher than those below (table 3).

Patients enrolled in the EUROPA trial primarily consisted of Caucasian males without overt heart failure, who were randomized to placebo or perindopril 8 mg daily. The generalizability of the presented results towards other patient groups, e.g. those with a higher proportion of women, heart failure, patients of other ethnicities, or those using other ACE-inhibitors or lower dosages of perindopril, may therefore be limited. Testing of these particular genetic variants in a large randomized heart failure trial would be required before suggesting the same phenomenon exists in that very different patient group.

Our combined primary endpoint consisted of cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest. Resuscitated cardiac arrest however only occurred in very few instances. Therefore our results with respect to clinical and pharmacogenetic determinants of treatment benefit are primarily associated with the incidence of cardiovascular mortality and non-fatal MI.

In order to facilitate clinical utility and ease of use, we specifically chose to develop an integer-based risk score. Disadvantages of integer-based risk scores in general include the fact that not all variables have exactly the same contribution to the model. Furthermore, certain combinations of risk factors may act synergistically to increase risk in a manner that is more than additive. Such synergy may be underestimated in a purely additive integer-based risk score.[29]

Replication of the three SNPs that formed the PGXscore in the PROGRESS trial[14] demonstrated concordant associations between the risk score and treatment benefit by perindopril.[12] The individual interaction terms of the three SNPs, however, did not reach statistical significance in that particular trial due to limited statistical power (replication could take place in 1051 patients only). Unfortunately larger replication cohorts are not available.

Although the clinical risk model consists of established cardiovascular risk factors, formally the combined clinical and pharmacogenetic risk score has not been independently validated on a separate dataset.

Finally, our risk model does not contain data on circulating serum biomarkers other than total cholesterol and creatinine levels. A prespecified substudy of the EUROPA trial, called PERTINENT, actually did study bradykinin, angiotensin II, but also markers of endothelial function (nitric oxide synthase) and inflammation (C-reactive protein, tumour

necrosis factor-α and von Willebrand factor). The use of perindopril was reflected in various circulating biomarker levels which were interpreted as a biochemical indication of normalization of the angiotensin II/bradykinin balance, reduction of inflammation and prevention of endothelial apoptosis.[24,25] Unfortunately, the cohort in which these biomarkers were assessed was too small in order to properly study the interaction between the various serum biomarkers and treatment effect by perindopril on clinical endpoints.

In conclusion, our results show that a combination of phenotypical and genetic information can be used to demonstrate a range of gradients of absolute treatment benefit by ACE-inhibitor therapy in an otherwise seemingly homogeneous population of patients with stable CAD. Clinical and pharmacogenetic profiling in individual patients may both clarify their distinct level of absolute risk of adverse events and furthermore also the degree of risk reduction by an ACE-inhibitor regimen. Refraining from ACE-inhibitor therapy in those patients that are expected to lack any treatment benefit may avoid unnecessary side-effects, reduce healthcare costs and increase overall efficacy of the drug. Future randomized clinical trials could advance the field of individualized medicine by incorporation of a similar combined clinical and pharmacogenetic approach in their study design.

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Conflict of interest disclosures

There is no commercial association that might pose a conflict of interest in connection with this manuscript. The sponsor of the EUROPA trial, Servier, had no role in the design, conduct, analysis or interpretation of this substudy, nor in the preparation, review or approval of this manuscript.

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ONLINE DATA SUPPLEMENT

Individualized ACE-inhibitor Therapy in Stable Coronary Artery Disease Based on Clinical and Pharmacogenetic Determinants;
The PERGENE risk model

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Online Table 1. The regression coefficients from the final multivariable model that were converted to the clinical risk score.

Variable	Regression coeeficients (ß)
Age (years)	0.023
Systolic bloodpressure (mmHg)	0.005
Creatinine clearance (ml/min)	–0.004
Total cholesterol (mmol/L)	0.125
Previous stroke or PAD	0.552
Male gender	0.447
Obesity (BMI>30 kg/m^2)	0.377
Current smoking	0.462
Symptomatic CAD	0.430
Diabetes mellitus	0.449
Previous MI	0.422
Family history of CAD	0.086
Previous revascularisation	–0.168

For the continuous variables (age, systolic bloodpressure, creatinin clearance and total cholesterol) the regression coefficients are described per unit of increase of that particular independent variable.

Online Table 2. Pharmacogenetic risk score on the basis of allele distribution

Angiotensin-II type I-receptor		Bradykinin type I receptor	**Pharmacogenetic Risk Score points**
SNP: Rs275651	Rs5182	Rs12050217	
AA	TT	AA	**0**
AT	CT	AG	**1**
TT	CC	GG	**2**

The range of pharmacogenetic risk scores = 0-6. Risk alleles for decreased benefit of perindopril treatment were T, C, and G for Rs275651, Rs5182 and Rs12050217 respectively. Points for each of the three separate SNPs need to be added to each other. E.g. a patient with AA, CC, AG (for Rs275651, Rs5182 and Rs12050217 respectively) has an individual pharmacogenetic risk score of 0+2+1 =3

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PRIMARY PCI DURING OFF-HOURS IS NOT RELATED TO INCREASED MORTALITY

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ABSTRACT

Aim: Previous studies have shown contradictory outcomes in ST-segment elevation myocardial infarction (STEMI) patients who underwent primary percutaneous coronary intervention (pPCI) during off-hours versus regular 'office' hours. We aimed to evaluate the relationship between pPCI timing (off-hours versus regular hours) and mortality in patients with STEMI undergoing pPCI.

Methods: The study population comprised 4352 consecutive STEMI patients treated with pPCI in a high-volume centre with a 24/7 programme during 2000–2009. Descriptive statistics and multivariable survival analyses were applied to evaluate the relationship between treatment during off-hours (Monday–Friday, 6.00 pm–8.00 am and weekends) versus regular hours and the incidence of all-cause mortality at 30-day and 4-year follow-up.

Results: A total of 2760 patients (63.4%) were treated during off-hours and 1592 patients (36.6%) during regular hours. With the exception of smoking, diabetes mellitus, use of glycoprotein IIb/IIIa antagonists and calcium antagonists, no major differences in baseline characteristics were observed between the groups. Mortality at 30-day follow-up was similar in patients treated during off-hours and those treated during regular hours (7.7% vs 7.7%; hazard ratio adjusted for potential confounders 1.03; 95% CI 0.82–1.28). Four-year mortality was similar (17.3% vs 17.3%; adjusted hazard ratio 0.95; 95% CI 0.81–1.11).

Conclusion: In STEMI patients who present during off-hours in a high-volume centre with 24/7 service, pPCI provides similar survival as patients who were treated during regular hours.

INTRODUCTION

Randomised clinical trials have convincingly demonstrated that patients with ST-segment elevation myocardial infarction (STEMI) who undergo primary percutaneous coronary intervention (pPCI) have better event-free survival and clinical outcomes than those treated with fibrinolysis.[1] In order to fully benefit from the instantaneous and long-term effects of pPCI, patients need to be treated as soon as possible after symptom onset. Guidelines recommend that pPCI be performed within 90 minutes of the first medical contact.[2–3] This recommendation is supported by several studies that reported a direct relation between (increased) time delay to pPCI and (worse) clinical outcome.[4–6] Short onset-to-treatment times are best guaranteed in hospitals with an established interventional cardiology programme that offers full service 24 hours per day, 7 days per week (24/7 programme).[2–3]

Since the occurrence of STEMIs are more or less randomly distributed over time, in a 24/7 programme, most patients will be treated during 'off-hours' – evening and night shifts and weekends. Previous studies have shown contradictory outcomes in STEMI patients who underwent pPCI during off-hours versus regular 'office' hours. However, most of these studies were conducted in centres using both fibrinolysis and pPCI for treatment of patients with STEMI, and did not evaluate long-term outcomes.[7–18] In the year 2000, pPCI became the standard treatment for STEMI in our institution and a 24/7 programme was established. Baseline, procedural and follow-up data of all patients undergoing PCI in our institution were systematically collected. Consequently, we were able to evaluate the relationship between pPCI timing (off-hours versus regular hours) and short- and long-term outcome in STEMI patients.

METHODS

Patient population

The Erasmus MC is a tertiary referral and teaching hospital in the broader region of Rotterdam (approximately 1.9 million inhabitants), located on the North bank of the Maas River. Between January 2000 and June 2004, the Erasmus MC was the only hospital in the region with pPCI facilities. From July 2004 onwards, the Maasstad hospital (also located in Rotterdam, on the south bank of the Maas River) started a 24/7 programme locally and provided regional pPCI service on Mondays and Thursdays and the first weekend of every month to improve service. The Erasmus MC provided pPCI service on the remaining days.

All consecutive patients 18 years or older who presented within 12 hours of symptom onset with ST-segment elevation myocardial infarction (STEMI) and who subsequently

underwent pPCI in our institution between January 2000 and December 2009 were included in the analysis. STEMI is defined as patients presenting with ischaemic symptoms and persistent (>20 min) ST-segment elevation in at least two contiguous precordial leads or at least two adjacent limb leads by ECG.[3] In total, 4541 pPCIs in 4352 patients were performed. In patients who were admitted more than once for pPCI (*n* =189), only the initial procedure was used for this analysis.

Patient management

Patient management was in accordance with the applicable guidelines of the European Society of Cardiology (ESC). Patients received an aspirin and a loading dose of clopidogrel (300–600 mg) before pPCI and preferably in the ambulance. Clopidogrel (75 mg/day) was given for at least one month in patients treated with bare metal stents (BMS), at least 3 months for patients treated with sirolimus-eluting stents (SES) and at least 6 months in patients treated with paclitaxel-eluting stents (PES) or everolimus-eluting stents (EES). After the procedure, all patients were advised to remain on aspirin (>80 mg/day) indefinitely. Periprocedural glycoprotein IIb/IIIa antagonists were left to the discretion of the treating interventional cardiologist.

Since 2000, the interventional cardiology department has the policy of using one particular stent as default in a given time interval. The default stent between January 2000 and April 2002 was a BMS, between April 2002 and March 2003 a SES, between March 2003 and March 2007 a PES, and an EES since March 2007. Of note, during the study period a small number of STEMI patients was treated with another stent due to participation in a clinical trial comparing stents.

Data collection

According to the approved standard data-management procedures in our department, data are collected on demographics, cardiovascular history, clinical risk factors and treatment characteristics for all patients undergoing PCI and are stored in an electronic database. Data elements are filled out immediately after the completion of the PCI by the interventional cardiologist and the technician who assisted during the procedure. The database, which is maintained by a dedicated IT officer, is mainly designed for administrative purposes. A systematic evaluation of data completion and data integrity is implemented for data that are used for research purposes.

Data management and follow-up

Mortality data related to the entire cohort was obtained from interrogation of municipal civil registries between April and September 2011. A health questionnaire was subsequently sent to all living patients with specific inquiries on rehospitalisation and major adverse cardiovascular events (MACE). For patients who had adverse events at

other centres, medical records or discharge summaries from the other institutions were systematically reviewed. General practitioners, referring cardiologists and patients were contacted in case further information was required.

Endpoint definitions

The primary endpoints were early mortality, which was defined as all-cause mortality within 30 days of the index event, and late mortality, which includes all-cause mortality at 1-year and 4-year follow-up. The secondary endpoints included repeat PCI (rePCI), coronary artery bypass grafting (CABG) or recurrent MI (reMI) and the composite endpoint of reMI, revascularisation (rePCI or CABG) and all-cause mortality at 30-day, 1-year and 4-year follow-up. ReMI at follow-up was diagnosed by recurrent typical clinical symptoms, the development of ST-segment elevation or left bundle branch block on electrocardiography with a CK-MB rise of three times the upper limit of normal and/or positive troponin levels in laboratory values. rePCI was defined as a repeat percutaneous intervention of any lesion located in the epicardial vessels. CABG was defined as a surgical intervention of any lesion located in the epicardial vessels.

Statistical methods

Off-hours were defined as weeknights (Monday to Friday from 6.00 pm to 8.00 am) and weekends (from Friday 6.00 pm to Monday 8.00 am).

Continuous variables are presented as mean ± standard deviation and categorical variables are expressed as numbers and percentages. Student's t-tests, Chi-square tests and Fisher's exact tests were applied to evaluate differences in baseline variables between patients treated during off-hours and regular hours, as appropriate.

We intended to obtain complete information in all patients, but failed to do so for the medication at discharge. Using missing value analysis (MVA) we evaluated the extent of missing data and searched for patterns of missing data. MVA showed that there were 6.2% missing values on average for medication at discharge. For the patients with at least one of the variables of interest missing, we decided to impute the missing values by multiple imputation.[19]

The incidence of events over time was studied with the use of the Kaplan-Meier method, whereas log-rank tests were applied to evaluate differences between the treatment groups (treatment during off-hours versus regular hours). Patients lost to follow-up were considered at risk until the date of last contact at which point they were censored.

Cox proportional hazard (PH) regression models were applied to evaluate the relationship between treatment during off-hours versus regular hours and the incidence of all-cause death at 30 days, 1 year and 4 years. The baseline clinical and procedural characteristics that are listed in Table 1 were considered as potential confounders for the 1-year and 4-year mortality analysis. As the number of events was limited at 30 days, we

Table 1. **Baseline and procedural characteristics according to pPCI timing.**			
	Off-hours *n* =2760	Regular hours *N* =1592	*p*-value
Age (years ±SD)	60.9±12.8	61.5±12.5	0.14
Male (%, *n*)	73.5, 2029	75.2, 1197	0.23
Medical history			
Hypertension (%, *n*)	40.1, 1107	38.6, 614	0.32
Hypercholesterolaemia	72.4, 1998	71.4, 1137	0.49
Diabetes mellitus (%, *n*)	13.0, 358	10.4, 165	0.011
Family history (%, *n*)	28.6, 790	27.6, 439	0.46
Current smokers (%, *n*)	41.8, 1154	36.0, 573	<0.001
Previous MI (%, *n*)	12.1, 334	13.1, 209	0.32
Previous PCI (%, *n*)	9.2, 147	7.9, 217	0.12
Previous CABG (%, n)	2.4, 66	3.2, 51	0.11
Renal impairment (%, n)	2.1, 57	1.8, 29	0.58
Procedural characteristics			
Cardiogenic shock (%, *n*)	4.9, 135	4.8, 76	0.86
Vessel disease			0.63
1-vessel disease (%, *n*)	54.8, 1513	55.2, 878	
2-vessel disease (%, *n*)	26.9, 742	27.6, 440	
3-vessel disease (%, *n*)	18.3, 505	17.2, 274	
Multi-vessel disease (%, *n*)	45.2, 1247	44.8, 714	0.83
Treated vessel			0.35
Left main (%, *n*)	3.5, 97	4.5, 71	
RCA (%, *n*)	34.0, 938	35.3, 562	
LCX (%, *n*)	14.3, 396	13.4, 213	
LAD (%, *n*)	46.6, 1285	44.9, 715	
Graft (%, *n*)	0.6, 17	0.9, 15	
Glycoprotein IIb/IIIa antagonists (%, *n*)	16.5, 456	21.4, 340	<0.001
Discharge medication			
Aspirin (%, *n*)	90.1, 2599	90.2, 1338	0.91
Calcium antagonist (%, *n*)	14.5 378	19.8, 294	<0.001
Beta-blockers (%, *n*)	51.6, 1342	54.0, 801	0.14
RAAS-inhibitors (%, *n*)	41.7, 1085	39.9, 592	0.25
Statins (%, *n*)	71,6, 1861	71.2, 1056	0.79

were only able to adjust for the following clinically relevant factors: age, sex, multi-vessel disease, shock, previous MI, renal impairment and diabetes mellitus.

Final results are presented as adjusted hazard ratios (aHR) with 95% confidence interval (CI). All statistical tests were two-tailed and a *p*-value <0.05 was considered significant. Statistical analyses were performed with SPSS for Windows version 17.0 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Key characteristics

Between 1 January 2000 and 31 December 2009, a total of 4352 consecutive patients presenting with STEMI underwent pPCI in our institution. A total of 2760 patients (63.4%) were treated during off-hours and 1592 patients (36.6%) during regular hours. Key characteristics of the two cohorts are presented in Table 1. With the exception of diabetes mellitus (13.0% vs 10.4%, $p = 0.011$), current smoking (41.8% vs 36.0%, $p < 0.001$) and use of glycoprotein IIb/IIIa antagonists (16.5% vs 21.4%, $p < 0.001$), no statistically significant differences in baseline and procedural characteristics were observed between the groups. It is noteworthy that the percentage of patients presenting with cardiogenic shock was similar in both groups (4.9% and 4.8%, respectively, $p = 0.86$). There were also no statistically significant differences in discharge medication, except for the use of calcium antagonists (14.5% vs 19.8%, $p < 0.001$).

Mortality

Information on survival status at 1-year follow-up was complete for 96.5% of patients. The median follow-up period was 1246 days (IQR 651–2228 days).

The cumulative incidence of all-cause mortality at 30-day follow-up was similar in the patients treated during off-hours and those treated during regular hours 7.7% vs 7.7% respectively (Kaplan Meier estimates). Similarly, no statistically significant differences were observed in all-cause mortality at 1-year (10.9% vs 12.5%) and 4-year follow-up (17.3% vs 17.3%). In fact, the cumulative incidence curves were superimposed throughout the entire 4-year follow-up period (Figure 1A). Multivariable adjustment for potential

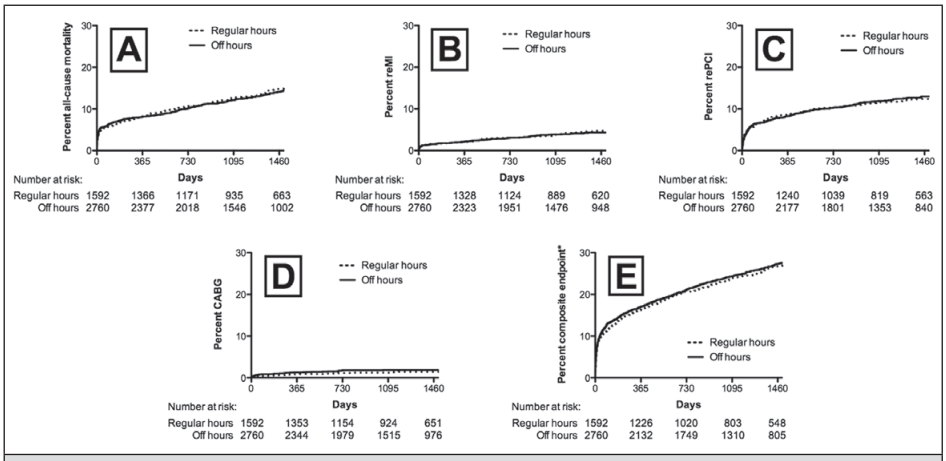

Figure 1. Clinical outcomes according to regular vs off hours. (A) all-cause mortality, (B) reMI, (C) rePCI, (D) CABG, (E) composite endpoint of all-cause mortality, reMI, rePCI and CABG.

Table 2. Clinical outcomes according to pPCI timing.

		30-day				1-year				4-year			
		Number of events *n*	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI	Number of events *n*	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI	Number of events *n*	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI
Death	Regular hours	123	7.7	1	1	173	10.9	1	1	251	17.3	1	1
	Off-hours	218	7.7	1.03 (0.82–1.28)	1,05 (0.84–1.31)	291	12.5	0.97 (0.81–1.18)	0.96 (0.79–1.16)	408	17.3	0.92 (0.65–1.18)	0.96 (0.81–1.12)
MI	Regular hours	25	1.6	1	1	43	2.9	1	1	40	5.5	1	1
	Off-hours	40	1.5	0.92 (0.56–1.52)	0.91 (0.55–1.50)	63	2.4	0.84 (0.57–1.24)	0.89 (0.60–1.31)	103	4.6	0.85 (0.63–1.16)	0.88 (0.65–1.19)
CABG	Regular hours	5	0.3	1	1	15	1.0	1	1	22	1.6	1	1
	Off-hours	16	0.6	1,85 (0.68–5.04)	2.06 (0.74–5.72)	35	1.4	1.35 (0.73–2.47)	1.51 (0.82–2.79)	46	3.0	1.22 (0.73–2.02)	1.39 (0.83–2.33)
rePCI	Regular hours	72	4.9	1	1	136	9.2	1	1	177	12.9	1	1
	Off-hours	112	4.2	0.88 (0.65–1.18)	0.87 (0.64–1.17)	217	8.6	0.92 (0.74-1.14)	0.94 (0.76–1.17)	304	13.4	0.99 (0.83-1.20)	1.03 (0.86-1.25)
Composite endpoint[a]	Regular hours	200	12.6	1	1	310	19.6	1	1	432	29.5	1	1
	Off-hours	339	12.3	0.98 (0.82–1.16)	0.99 (0.83–1.18)	538	19.6	1.00 (0.87-1.15)	1.03 (0.89–1.18)	741	29.5	0.98 (0.87-1.10)	1.046 (0.93-1.18)

[a]Composite endpoint of reMI, revascularisation and all-cause mortality.

confounders of the relation between treatment timing and the incidence of all-cause mortality did not change this finding (aHR at 4 years 0.96 and 95% CI 0.81–1.12).

Non-fatal and composite endpoints

We did not find any clinically relevant difference in the incidence of non-fatal endpoints between patients treated during off-hours vs regular hours. Results were similar at short-term and long-term follow-up (Table 2, Figure 1B–D). At 4-year follow-up, the crude cumulative incidences of reMI were 5.5% and 4.6%, the incidences of CABG were 1.6% and 3.0%, and the incidences of rePCI were 12.9% and 13.4%, respectively. Again, cumulative incidence curves were superimposed for all these endpoints. Treatment timing had no contribution in multivariable Cox PH models that related patient characteristics with non-fatal clinical outcomes. Adjusted HRs of treatment timing were not significant and close to 1 for all endpoints at all three follow-up times that we studied.

The cumulative incidence of the composite endpoint of all-cause death, reMI, PCI or CABG at 4-year follow-up was 29.5% in patients treated during off-hours and 29.5% in those treated during regular hours (Table 2, Figure 1E). The aHR was 1.05 and the 95% CI ranged from 0.93 to 1.18, indicating that there was no association between treatment timing and the incidence of this composite endpoint.

Findings in subgroups

Figure 2 shows the relationship between treatment timing and 30-day all-cause mortality in a number of clinically relevant subgroups. All in all, in the subgroups that we considered, we found no major deviations from the overall result. Except in the category relating to the history of MI, 95% confidence intervals of treatment effect were largely overlapping, and none of the formal heterogeneity tests was statistically significant.

DISCUSSION

In this long-term follow-up study of STEMI patients who were treated during 2000–2009 in a high-volume centre with 24/7 service, clinical outcomes were similar in patients undergoing pPCI during off-hours and during regular hours. This similarity in outcome was already observed at 30 days and was maintained until 4 years after the initial procedure. Consistent results were seen in clinically relevant subgroups, including the elderly and patients with multi-vessel disease.

The quality of care delivered to STEMI patients may differ during day and night because of variations in door-to-balloon time, performance of physicians, catheterisation laboratory and coronary care staff. Compared to regular office hours, hospital staffing is generally reduced during nights and on weekends compared with weekdays. The

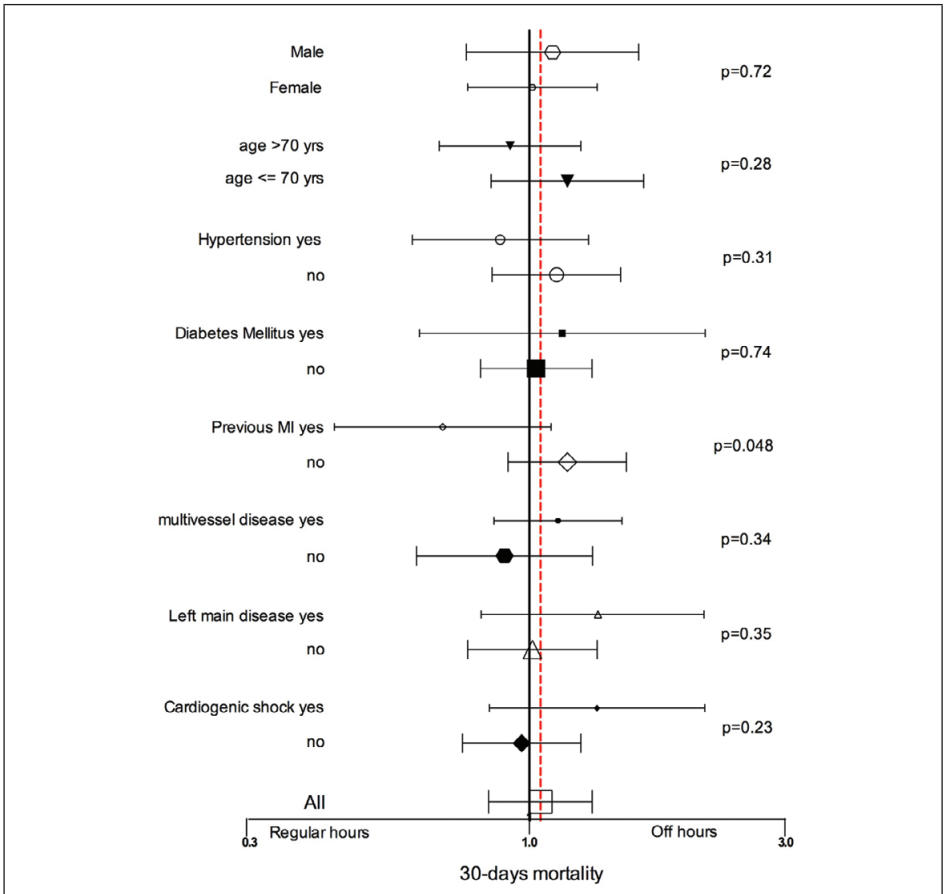

Figure 2. Treatment during off hours in different subgroups.

short-term outcome (after adjustment for any differences in case mix) may be regarded as a proxy measure of the quality of care during the procedure, whereas the long-term outcome to a greater extent depends on the development of the disease, the use of long-term medication and (probably) the use of coronary revascularisations. Interestingly, we found few differences in baseline characteristics between patients treated during off-hours and regular hours. Also medical treatment at discharge was similar. Apparently, in the region of Rotterdam, STEMI patients constitute a homogeneous population, regardless of the timing of presentation. Consequently, the point estimates of the effect of treatment timing did not change after adjustment for patient characteristics. The estimates of the effect for all the endpoints indicated that treatment during off-hours is as safe and effective as treatment during regular hours.

Previous studies have shown contradictory results in outcome in STEMI patients who underwent pPCI during off-hours versus regular hours. Whereas some studies showed

that presentation and treatment during off-hours only had limited impact on in-hospital mortality,[7,9,12,13,15,16,18] other studies revealed higher in-hospital mortality in pPCI patients during off-hours than regular hours.[8,10,11,14] A straightforward comparison of these clinical studies is complicated by differences in patient characteristics and (medical) co-treatment, as well as by the applied definitions. For example, Henriques et al, who reported higher mortality after off-hour treatment, focused on the circadian patterns of symptom onset, including routine duty hours (0800–1800) versus off-hours irrespective of day of the week.[8] In the study by Kostis et al,[10] the significant difference in 30-day mortality for patients treated during off-hours disappeared after adjustment for the use of invasive cardiac procedures, which appeared less often in patients admitted at weekends. Furthermore, in some of the previous studies, patients were not only treated with pPCI, but also with fibrinolysis, which may have led to different results.[9–11,15,18] Since 2000 in our centre, pPCI is the default strategy for all STEMI patients on a 24/7 basis, precluding potential bias due to fibrinolysis. On the other hand, most patients treated in our centre are referred for pPCI by hospitals in the larger region of Rotterdam that do not provide a 24/7 pPCI service. Although, apparently, the referral pattern is not dependent on the day of the week and the time of the day (as we found no differences in patient characteristics in relation to treatment timing), the patient selection by referral hospitals might partly explain the consistent outcomes.

Study limitations

Other reported predictors of outcome after pPCI include time delay from symptom onset to the first balloon inflation[4–6] and physician volume.[20,21] Since the database that we used for our study was not specifically designed to address these issues, several relevant quality parameters have not been recorded prospectively. Still, we retrospectively recorded the time between hospital admission and the start of the pPCI procedure (data were available for 70% of patients), and we found no differences between patients treated during off-hours versus those treated during regular hours (data not shown). The presented results are based on a single-centre experience, which limits the external validity. Nevertheless, the Thoraxcenter Rotterdam can be considered representative for larger tertiary referring and teaching (academic) hospitals in Western populations.

For the follow-up on non-fatal endpoints we were dependent on the responses of patients on health questionnaires that were systematically sent to all living patients, with specific inquiries on rehospitalisation and MACE. Thus, we might have missed some non-fatal endpoints, particularly those that did not result in hospital admissions. We have no indication that under-reporting (if any) was related to the timing of the initial treatment. This phenomenon might have resulted in effect estimates that are biased towards the null. Still, we are confident that similar effects were seen for all ('hard' and 'softer') endpoints.

CONCLUSION

STEMI patients who were treated in a high-volume centre with a 24/7 programme have similar short- and long-term outcomes whether they are treated during off-hours or regular hours. Our findings, which are based on systematic monitoring of treatment outcome results, do not necessitate us changing our practice. Instead, these results may encourage other centres to expand their service.

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Conflict of interest

None declared by authors.

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CORONARY VULNERABILITY

V

EPILOGUE

CORONARY VULNERABILITY

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SUMMARY AND CONCLUSIONS

***Part 1**, "**Vulnerable Blood**",* focusses on the additional value of several serum biomarkers for the prediction of MACE on a relatively long term (4 to 10 years of follow-up). These markers are traditionally measured once at the start of follow-up and hence assumed to reflect a constant cardiovascular risk, in a similar way as traditional risk models incorporate clinical risk factors.

Chapter 2 describes a multimarker model in which Troponin (Tn), Interleukin-10, myeloperoxidase and placental growth factor predict 4-year MACE rates in 1090 patients with non ST-elevation acute coronary syndrome. This model was able to stratify a seemingly homogenous study population into a relative low risk (6.0% event rate when all markers were normal) to a very high-risk subgroup in which the MACE rate was 35.8% when three of four biomarkers were abnormal.

Chapters 3 and 4 focus on patients undergoing a percutaneous coronary intervention and conclude that C-reactive protein (CRP) is associated with long-term MACE. Lipoprotein A may be of interest with respect to short-term prognosis after PCI.

***Part 2**, "**Vulnerable Period**",* focusses on serum biomarkers as well, but here the train of thought is to capture the dynamics of coronary pathophysiology, i.e. that the risk of MACE within an individual patient is not constant, but variable over time. Hence repeated biomarker measurements are explored in the BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS), in order to evaluate whether fluctuations in biomarker levels can predict the risk of an imminent MACE within the days to weeks to come.

Chapter 5 describes the rationale and study design of the multicenter, prospective BIOMArCS study, together with the baseline clinical characteristics of the 844 enrolled patients presenting with ACS, either with or without ST-elevation and at least one additional cardiovascular risk factor.

The paradigm of the *vulnerable period* is based on the concept that individual patients with CAD actually do not have a constant risk over time. Long periods of stability, with minimal plaque progression and low risk of CV events, are alternated by periods of increased plaque instability and rapid plaque progression, during which the risk of sudden plaque disruption and thrombotic coronary occlusion within short time spans is high. This is a complex and multifactorial pathophysiological process in which temporal variations in distorted lipid metabolism, vascular inflammation, endothelial dysfunction, increased thrombogenicity and myocardial ischaemia play an important role. In order to be able to capture a signal of changing risks over time, frequently repeated measurements are required of markers that reflect the above-mentioned pathophysiological

processes. In BIOMArCS venapuncture was performed every 2 weeks during the first six months and every month thereafter during 1-year follow-up in order to evaluate the obtained repeated biomarker information for risk prediction.

As per the inclusion criteria, every BIOMArCS patient had endured an index ACS in order to qualify for enrollment. Hence it is of great importance to understand the Tn release patterns and stabilization after the index ACS prior to any further evaluation of this marker. **Chapter 6** describes the Tn washout patterns as measured with current high-sensitivity assays. Troponin levels stabilized in approximately 2 weeks after the index event. Intriguingly, low individual variation but large between-patient differences were observed thereafter. Using the first samples taken one month after ACS, we were able to compose a patient specific reference value for approximately 80% of the patients with just two measurements.

Chapter 7 describes the primary results of BIOMArCS through an evaluation of repeated measurements of high-sensitivity Troponins, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and high-sensitivity C-reactive protein. In this cohort of asymptomatic post ACS patients with preserved ventricular function and guideline based treatment for secondary prevention, we observed that those patients with persistently elevated Tn and NT-proBNP levels were at a significantly higher risk of MACE during 1-year follow-up. In addition, 20% of the patients had asymptomatic, isolated TnI peaks of 10 pg/mL, which was associated with a 2.9 fold increased risk of MACE during follow-up. Similarly an asymptomatic, isolated CRP peak of 10 mg/L, was also associated with a 2.9 fold increased risk of MACE during follow-up.

In ***part 3***, *"**Vulnerable Plaque**"*, the centre of interest is around invasive coronary imaging (coronary angiography, intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS)) for the prediction of MACE, as well as cross-sectional analyses evaluating the relation between these imaging techniques and serum biomarkers.

Chapter 8 describes the design and rationale of ATHEROREMO, the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis study. This large, prospective, observational cohort study was designed to evaluate the relation between novel circulating biomarkers, coronary plaque characteristics as determined by intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) and clinical outcome. IVUS and NIRS imaging was performed in a 40 mm long non-stenotic segment of a nonculprit coronary artery in patients referred for angiography due to stable angina pectoris or ACS.

Chapters 9 and 10 evaluate the prognostic value of the novel intracoronary imaging modality known as near-infrared spectroscopy. This technique is capable of assessing lipid core-containing plaques, which can subsequently be quantified as a lipid core burden index (LCBI). Patients with a relatively high LCBI had a 4-fold risk of MACE during 1-year follow-up (**chapter 9**). A similar association was observed when follow-up was extended to 4 years (**chapter 10**).

Chapters 11 and 12 describe the prognostic value of IVUS assessments of non-stenotic segments during 1 and 4 years of follow-up in 581 patients. Similarly to the NIRS findings, the overall conclusion here is that a higher amount of plaque burden within the coronary vessel wall is predictive of higher MACE rates. In addition, these NIRS and IVUS studies confirm that imaging of a relatively short and angiographically non-stenotic segment does indeed provide prognostic information, thus suggesting that the vessel wall characteristics in these segments seem to reflect a global measure of intracoronary disease burden, despite being short and relatively unobstructed.

Whereas chapters 9-12 rely on a detailed characterization of a short segment of the coronary vessel wall, **chapter 13** focusses on the predictive value of lumen characteristics as obtained by coronary angiography of all three epicardial vessels. The SYNTAX score II, a long-term mortality prediction model for patients with left main and/or three vessel disease, is evaluated in the setting of only one- and two-vessel disease. Syntax score II also proved to be an independent significant predictor for 4.5-year mortality in this non-left main or three vessel disease cohort.

The interlink between chapters 9-13 is that the anatomy of coronary atherosclerosis, whether it is obtained from vessel wall characterization or lumenography, contains prognostic information even after adjustment for those traditional clinical risk factors that form the backbone of current risk prediction models for patients with coronary artery disease.

The following chapters 14-19 describe the crosslink between the *vulnerable plaque* and *vulnerable blood*; the associations between coronary plaque characteristics and serum biomarkers are evaluated. With our epidemiologic studies, we do not aim to unravel the biologically mechanistic intricacies of the interplay between serum proteins and coronary plaque. Nevertheless, it is intriguing to observe that serum biomarkers as C-reactive protein (**chapter 14**) and Troponin (**chapter 15**), that have been described as independent predictors for MACE in Parts 1 and 2 of this thesis (in **chapters 2, 3, 4 and 7** respectively), are also associated with IVUS plaque characteristics. In the following chapters, we describe the relation between plaque characteristics and serum proprotein

convertase substilisin/kexin type 9 (**chapter 16**), serum chemokines (**chapter 17**), serum cytokines (**chapter 18**) and Von Willebrand factor (**chapter 19**). Finally, **chapter 20** is somewhat different as it does not evaluate a potentially fluctuating serum biomarker, but a genetic trait; haptoglobin polymorphisms are evaluated in relation to coronary plaque characteristics.

Accurate risk prediction is important to understand future risks of CAD patients, but clearly prediction alone will not alter the outcome. For that purpose, intervention studies are required in those deemed at high risk. Such studies, often combined with the search for those patient subsets to derive most benefit from the interventions, are described in ***Part 4***, *"**Intervention Studies**"*.

Chapter 21 is part of the Integrated Biomarker and Imaging Study 2 (IBIS 2), and describes the effects of a lipoprotein-associated phospholipase A2 inhibitor on repeatedly measured Tn levels.

Chapter 22 describes the main outcome of the IBIS 3 study, a prospective, non-randomized trial in which the effect of high intensity statin therapy is evaluated through repeated IVUS (n=164) and NIRS (n=103) measurements of a non-stenotic segment of a nonculprit coronary artery at baseline and after 1 year follow-up. High intensity rosuvastatin therapy resulted in a neutral effect on necrotic core and LCBI.

Chapters 23 and 24 describe 8726 patients enrolled in the randomized, placebo controlled EUROPA trial, which evaluated the efficacy of perindopril in patients with stable CAD. In **chapter 23**, models are constructed to evaluate the treatment effect of perindopril in terms of 5 year absolute risk reduction for MACE, based on clinical variables (Europa score) and clinical and pharmacogenetic variables (Europa-GEN score). **Chapter 24** describes the PERindopril GENEtic (PERGENE) risk model, which incorporates clinical and pharmacogenetic variables. On the basis of this cost-effective risk scoring model a very wide range of gradients of absolute treatment benefit on ACE-inhibitor therapy was demonstrated in a seemingly homogenous trial population. The PERGENE score could identify patient subgroups with an annual number needed to treat (NNT) as low as 29, as well as patients with an annual NNT as high as 521.

Finally **chapter 25** describes a single center, primary PCI experience in 4352 patients, and more specifically whether the outcome differs between those treated during regular office hours and those treated during so-called off-hours. Short and long-term mortality were similar in both groups.

In conclusion, this thesis focusses on patients with CAD. Despite current optimal medical therapy, their residual risk for MACE on a group level is evident. Equally evident is the observation that the persons that comprise this heterogenous group of CAD patients,

differ in their individual risk profile. Serum biomarkers, coronary imaging techniques and (pharmaco)genetics can all be successfully deployed in order to aid further risk stratification, i.e. identification of those at a higher, or perhaps even very low risk of a coronary event. The selection of any one of these techniques differs from one instance to another, and depends on the formulation of the particular research question that needs to be answered.

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DUTCH SUMMARY
NEDERLANDSE SAMENVATTING

In het **eerste deel** van dit proefschrift, genaamd *"**Vulnerable Blood**"*, ligt de nadruk op de toegevoegde waarde van serum biomarkers ten behoeve van het voorspellen van cardiovasculaire uitkomsten op een relatief lange termijn (4-10 jaar) bij patiënten met coronairlijden. Deze markers worden doorgaans eenmalig gemeten bij aanvang van de follow-up. De aanname is dat een dergelijke eenmalige meting van een eiwit in het bloed een cardiovasculair risico reflecteert dat constant is over de tijd, zoals dat ook wordt aangenomen voor klinische risicofactoren zoals bijvoorbeeld diabetes of hypertensie etc. in traditionele risico modellen.

Zo wordt de associatie van serum biomarkers als Troponine, Interleukine-10, myeolperoxidase, placental growth factor, C-reactive protein (CRP) en lipoproteine A met cardiovasculaire uitkomsten op de lange termijn aangetoond.

Deel 2, *"**Vulnerabele Periode**"*, legt eveneens de nadruk op serum biomarkers, maar hier is het doel om de dynamiek van de coronaire pathofysiologie te pogen te detecteren middels kort op elkaar volgende, dus herhaalde, biomarker metingen. De gedachtengang is dat het risico op een hartinfarct in een individuele patiënt niet constant over de tijd is, maar dat het risico wisselt in de loop der tijd. In de *BIOMarker study to identify the Acute risk of a Coronary Syndrome* (*BIOMArCS*) is daarom aan 844 hartinfarct patiënten gevraagd om gedurende de loop van 1 jaar herhaald bloed af te geven (gemiddeld 17 keer). Uit deze herhaalde metingen blijkt dat patiënten met een chronisch verhoogde Troponine en NT-proBNP waarde, alsook hen die pieken in de concentratie van Troponine en CRP vertoonden, een hoger risico hebben op het primaire eindpunt, een combinatie van overlijden, hernieuwd hartinfarct of urgente coronaire revascularisatie procedures.

In **deel 3**, *"**Vulnerabele Plaque**"*, ligt de nadruk op invasieve afbeelding van de kransslagaders door middel van coronair angiografie, intravascular ultrasound (IVUS) en near-infrared spectroscopy (NIRS). Er wordt onder andere, voor het eerst, aangetoond dat de mate van cholesterol in de kransslagaders, zoals gedetecteerd met de op licht gebaseerde techniek NIRS, voorspellend is voor het optreden van overlijden, hartinfarct en urgente coronaire revascularisatie procedures.

Verder worden er cross-sectionele relaties aangetoond tussen intracoronair gemeten plaque karakteristieken en serum biomarkers zoals Troponine, CRP, serum proprotein convertase substilisin/kexin type 9 (PCSK9), serum chemokines, serum cytokines, Von Willebrand factor en ook met haptoglobine polymorfismen.

Accurate risico predictie voor het optreden van een hartinfarct is uiteraard belangrijk, echter risico predictie alleen zal de uitkomsten niet verbeteren. Voor dat doel zijn "***Interventie Studies***" benodigd die in **deel 4** worden beschreven.

Zo wordt, onder andere, het effect van een lipoprotein-geassocieerde phospholipase A2 inhibitor op herhaalde Troponine metingen in de Integrated Biomarker and Imaging Study 2 (IBIS-2) beschreven.

In de IBIS-3 studie wordt door middel van herhaalde invasieve IVUS en NIRS metingen het effect van hoog-intensiteits statine therapie op de kransslagaders onderzocht.

Data uit de gerandomiseerde, placebo gecontroleerde EUROPA studie naar het effect van de ACE-remmer perindopril zijn gebruikt om grote verschillen in het behandeleffect aan te tonen. Deze verschillen in behandeleffect zijn afhankelijk van zowel klinische als genetische patiënt karakteristieken.

Als laatst wordt het effect van het tijdstip van spoed dotterprocedures bij hartinfarct patiënten onderzocht.

Samenvattend ligt de nadruk van dit proefschrift op risico stratificatie bij patiënten met coronairlijden. In deze groep patiënten is, ondanks de huidige optimale medische therapie, nog altijd een evident residueel risico op hartinfarct en cardiovasculaire dood aanwezig. Eveneens is het evident dat de personen binnen deze heterogene groep verschillen in het individuele risicoprofiel. Serum biomarkers, coronaire beeldvormingstechnieken en (farmaco)genetica kunnen allen succesvol ingezet worden voor verdere risico stratificatie, d.w.z. identificatie van patiënten met een hoger, of wellicht zelfs heel erg laag risico op een coronair event. De selectie van deze verschillende technieken voor risico stratificatie is afhankelijk van de formulering van de specifieke onderzoeksvraag.

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LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

47. **Oemrawsingh RM**, Akkerhuis KM, de Mulder M, Umans VA, Kietselaer B, Schotborgh C, Ronner E, Lenderink T, Liem A, Haitsma D, van der Harst P, Asselbergs FW, Maas A, Oude Ophuis AJ, Ilmer B, Dijkgraaf R, de Winter RJ, Kie The SH, Wardeh AJ, Hermans W, Cramer E, van Schaik RH, Hoefer IE, Doevendans PA, Simoons ML, Boersma E; BIOMArCS Investigators. *High-Frequency Biomarker Measurements of Troponin, NT-proBNP, and C-Reactive Protein for Prediction of New Coronary Events After Acute Coronary Syndrome*. **Circulation**. 2019 Jan 2;139(1):134-136.
46. Vroegindewey MM, **Oemrawsingh RM**, Kardys I, Asselbergs FW, van der Harst P,Oude Ophuis AJ, Etienne Cramer G, Maas A, Hong Kie The S, Wardeh AJ, Mouthaan H, Boersma E, Akkerhuis KM. *The temporal pattern of immune and inflammatory proteins prior to a recurrent coronary event in post-acute coronary syndrome patients*. **Biomarkers.** 2018 Dec 4:1-7.
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41. Vroegindewey MM, Schuurman AS, **Oemrawsingh RM**, van Geuns RJ, Kardys I, Ligthart J, Daemen J, Boersma E, Serruys PW, Akkerhuis KM. *SYNTAX score II predicts long-term mortality in patients with one- or two-vessel disease*. **PLoS One**. 2018 Jul 2; 13(7):e0200076.
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CORONARY VULNERABILITY

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PHD
PORTFOLIO

PHD PORTFOLIO SUMMARY

	Year	Workload (ECTS)
Research skills		
– NIHES Master of Science programme in Clinical Epidemiology	2010	70
		*
Presentations		
– Netherlands Heart Foundation Papendal course	2008	0.3
– Netherlands Heart Foundation Wetenschapsdag	2008	0.3
– Spring Congress Netherlands Society of Cardiology (NVVC)	2008	0.3
– Spring Congress Netherlands Society of Cardiology (NVVC)	2009	0.5
– European Society of Cardiology Congress (Barcelona)	2009	0.6
– American Heart Association Scientific Sessions (Orlando)	2009	0.3
– American College of Cardiology.10 (Atlanta)	2010	0.5
– World Congress of Cardiology (Beijing)	2010	1.5
– European Society of Cardiology Congress (Stockholm)	2010	0.3
– European Society of Cardiology Congress (Amsterdam)	2013	1.0
– American Heart Association Scientific Sessions (Dallas)	2013	0.3
– American College of Cardiology.14 (Washington)	2014	0.3
– European Society of Cardiology Congress (Barcelona)	2014	0.6
– Autumn Congress Netherlands Society of Cardiology (NVVC)	2014	0.5
– WCN Congress (Amsterdam)	2015	0.5
– Optics in Cardiology (Zurich)	2018	0.5
** 0.3 ECTS for a poster and 0.5 ECTS for an oral presentation*		
International conferences		
– European Society of Cardiology Congress (Barcelona)	2009	1.5
– American Heart Association Scientific Sessions (Orlando)	2009	1.2
– American College of Cardiology.10 (Atlanta)	2010	1.2
– World Congress of Cardiology (Beijing)	2010	1.2
– European Society of Cardiology Congress (Stockholm)	2010	1.5
– European Society of Cardiology Congress (Amsterdam)	2013	1.5
– American Heart Association Scientific Sessions (Dallas)	2013	1.2
– American College of Cardiology.14 (Washington)	2014	1.2
– European Society of Cardiology Congress (Barcelona)	2014	1.5
– European Society of Cardiology Congress (London)	2015	1.5
– ACC New York Cardiovascular Symposium	2015	1.2
– European Society of Cardiology Congress (Rome)	2016	1.5
– EuroPCR incl fellows course (Paris)	2017	1.5
– European Society of Cardiology Congress (Barcelona)	2017	1.5
– Optics in Cardiology (Zurich)	2018	0.9

	Year	Workload (ECTS)
– EuroPCR incl fellows course (Paris)	2018	1.5
– CSI Frankfurt 2018	2018	1.2
– European Society of Cardiology Congress (Munich)	2018	1.5
Seminars and workshops		
– Netherlands Heart Foundation Papendal course	2008	2.0
– Netherlands Heart Foundation Wetenschapsdag	2008	0.2

CORONARY VULNERABILITY

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CORONARY VULNERABILITY

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ABOUT THE AUTHOR
CURRICULUM VITAE

ABOUT THE AUTHOR

Rohit Mansingh Oemrawsingh was born in The Hague, The Netherlands, on the 23rd of May 1980. After graduating high school from the Christelijk Gymnasium Sorghvliet, he studied medicine at the University of Leiden and obtained his MD degree in 2007. During this period he also served as founding chairman and secretary of *Global Human Rights Defence*, a nowadays professional human rights organisation. The final stages of his medicine training (senior internship) were conducted at the Thoraxcenter of the Erasmus University Medical Center in Rotterdam, where he subsequently was appointed to a preset programme in which a PhD fellowship and a clinical residency and fellowship in cardiology were intertwined during a 9,5 year time course.

His first three years of research under the supervision of Prof. Eric Boersma and Dr. Martijn Akkerhuis primarily focused on the inception of the BIOMArCS study as described in this thesis. The biomarker study platform, as developed from scratch during that time, was subsequently successfully replicated for the use of several other studies, such as the ATHEROREMO and IBIS-3 studies, that focused on the relationship between biomarkers and intracoronary imaging modalities, as also described in this thesis.

His internal medicine residency was performed at the Sint Franciscus Gasthuis (programme director Dr. Arie P. Rietveld). After another dedicated year of research at the Clinical Epidemiology Unit of the Thoraxcenter, he completed his cardiology training in Breda (Amphia Hospital, programme director Dr. Marco A. Alings) and Rotterdam (Erasmus MC, programme directors Dr. Folkert ten Cate, Prof. Jaap W. Deckers and Dr. Tjebbe W. Galema). During this period he frequently presented his research results at international venues. In addition, he was awarded a Young Investigator Award in Coronary Pathophysiology and Microcirculation during the European Society of Cardiology Congress 2013 and won a second prize during the 2e WCN Onderzoeksprijs event organized by the Werkgroep Cardiologische Centra Nederland.

Subsequently, his interventional cardiology fellowship was conducted under the supervision of the team of interventional cardiology consultants led by Dr. Peter den Heijer at the Amphia Hospital in Breda.

As of September 2018, Rohit serves as an interventional cardiologist at the Albert Schweitzer Hospital in Dordrecht with an unremitting research interest in coronary pathophysiology and interventional cardiology.