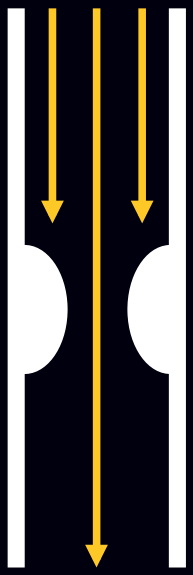
CORONARY ARTERY DISEASE

FROM ATHEROSCLEROSIS TO CARDIOGENIC SHOCK

Jin Ming Cheng



Coronary Artery Disease: from Atherosclerosis to Cardiogenic Shock

Jin Ming Cheng

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Promotor: Prof.dr. H. Boersma

Andere leden: Prof.dr. O.H. Franco
Prof.dr. G. Pasterkamp
Prof.dr. J.J. Piek

Copromotoren: Dr. K.M. Akkerhuis
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Voor mijn ouders

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INTRODUCTION

GENERAL INTRODUCTION

Coronary artery disease is projected to become the largest cause of disease burden worldwide. To lower this disease burden, it is important to improve risk prediction in patients with coronary artery disease. The prediction of a patient's risk for future cardiovascular events is likely to be more accurate when information on the patient's underlying coronary atherosclerotic disease can be taken into account. This thesis investigates two methods that may provide such patient-specific information, namely intracoronary imaging and blood biomarkers. Additionally, this thesis specifically focuses on risk prediction and management of patients with cardiogenic shock complicating acute myocardial infarction.

The aim of this thesis is twofold:

1. to investigate the prognostic value of intracoronary imaging and blood biomarkers in patients with coronary artery disease;
2. to improve risk prediction and management of patients with cardiogenic shock from acute myocardial infarction.

Intracoronary imaging provides patient-specific information on the extent and phenotype of coronary atherosclerosis, and may also be used to identify coronary lesions that are at high risk to rupture. Additionally, measurement of blood biomarkers provides patient-specific information on the ongoing pathophysiological processes of coronary atherosclerosis. In this thesis:

- we investigate the prognostic value of intravascular ultrasound virtual histology (IVUS-VH) imaging and near-infrared spectroscopy (NIRS) of coronary atherosclerosis in patients with coronary artery disease;
- we measure biomarkers of myocardial necrosis, inflammation, lipid profile, and blood coagulation to investigate their association with coronary plaque characteristics assessed by intracoronary imaging, and to investigate their value to predict cardiovascular outcome in patients with coronary artery disease;
- we use novel large-scale biomarker measurement methods, including proteomics, lipidomics and multiplex assays, to reveal and/or validate novel biomarkers that have the ability to improve prediction of cardiovascular outcome in patients with coronary artery disease.

Cardiogenic shock is a life-threatening state of inadequate tissue perfusion due to dysfunction of the heart. It complicates 5-10% of all cases of acute myocardial infarction. Despite improvement in therapy over the last decades, it remains the leading cause of death in patients with acute myocardial infarction. The in-hospital mortality rate in

these patients has been reported to be as high as 50%. Improvement in risk prediction and management of cardiogenic shock is required to improve clinical outcome of these patients. In this thesis:

- we develop a risk chart for early assessment of 30-day mortality risk, which may aid clinical decision making in the acute phase;
- we investigate the use of sublingual microcirculation measurements and its value to predict 30-day outcome;
- we perform a meta-analysis to compare the effects of percutaneous left ventricular assist devices with that of intra-aortic balloon pump (IABP) counterpulsation on hemodynamics and 30-day outcome;
- we evaluate the order of IABP insertion and primary percutaneous coronary intervention (PCI), and its association with outcome;
- we evaluate the long-term outcome of patients who were treated with IABP for cardiogenic shock from acute myocardial infarction;
- we review the current role of IABP counterpulsation in acute myocardial infarction and high-risk PCI in patients with or without cardiogenic shock.

Chapter 1

Rationale and design of the ATHEROREMO-IVUS study

De Boer SPM, Cheng JM, Garcia-Garcia HM, Oemrawsingh RM, Van Geuns RJ, Regar E, Zijlstra F, Laaksonen R, Halperin E, Kleber ME, Koenig W, Boersma E, Serruys PW.

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 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.

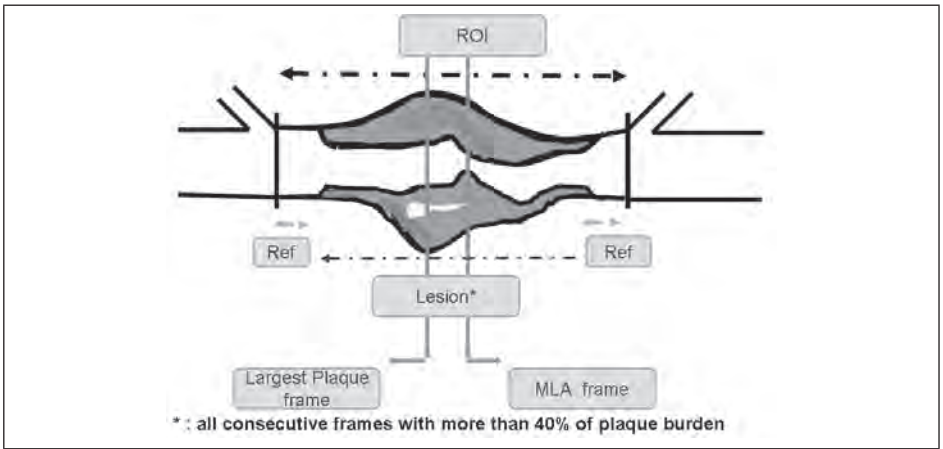

Figure 1. Methodology for detection of lesion and reference segments
MLA: minimal luminal area; REF: reference segments; ROI: region of interest.

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

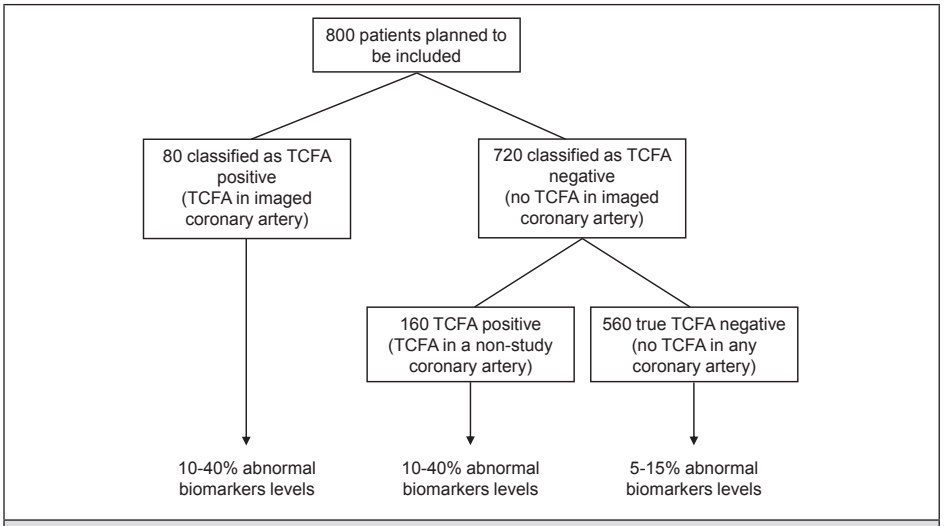

Figure 2. Expected prevalence of thin-cap fibroatheroma lesions
TCFA: thin-cap fibroatheroma.

(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 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 is 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 is 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

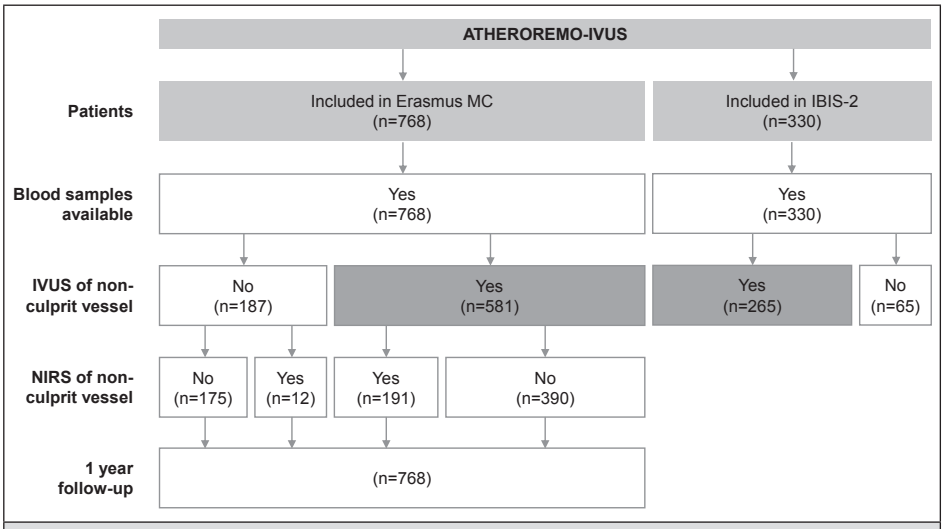

Figure 3. Actual patient inclusion
Finally, blood samples were stored for 1098 patients, IVUS of a non-culprit vessel was performed in 846 patients (grey shaded) and NIRS was performed in 203 patients.
IBIS-2: Integrated Biomarker and Imaging Study-2; IVUS: intravascular ultrasound; NIRS: near-infrared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

IMAGING OF CORONARY ATHEROSCLEROSIS

Chapter 2

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

Cheng JM*, Garcia-Garcia HM*, de Boer SP, Kardys I, Heo JH, Akkerhuis KM, Oemrawsingh RM, van Domburg RT, Ligthart J, Witberg KT, Regar E, Serruys PW, van Geuns RJ, Boersma E.

* These authors contributed equally.

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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.

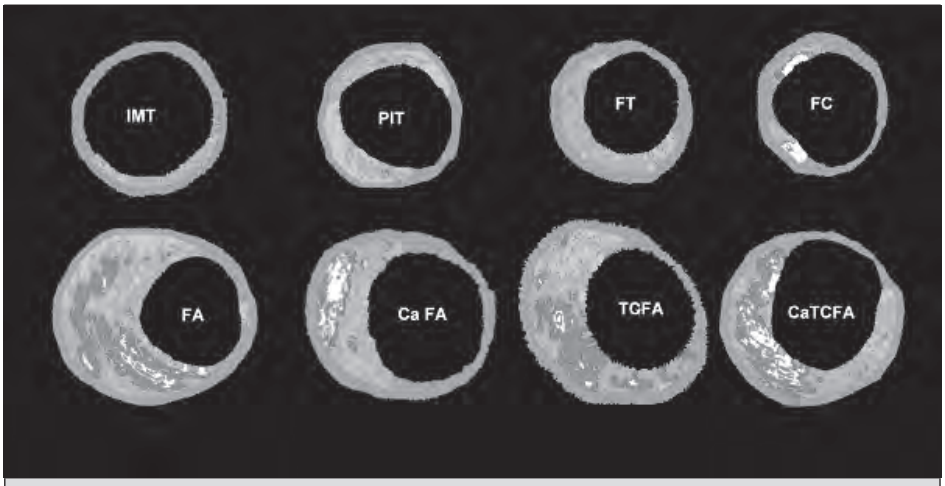

Figure 1. Classification of plaque morphology with intravascular ultrasound virtual histology
IMT, intimal medial thickening; PIT, pathological intimal thickening; FT, fibrotic plaque; FC, fibrocalcific plaque; FA, fibroatheroma; CaFA, calcified fibroatheroma; TCFA, thin-cap fibroatheroma; CaTCFA, calcified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

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.

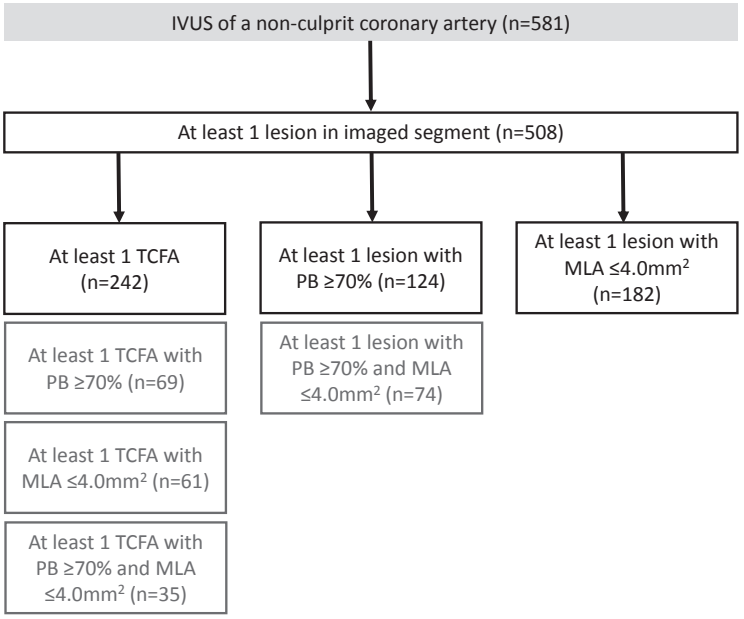

Figure 2. Study participants and presence of high risk coronary lesions on intravascular ultrasound
IVUS, intravascular ultrasound; MLA, minimal luminal area; PB, plaque burden; TCFA, thin-cap fibroatheroma.

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 major adverse cardiac events, 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

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

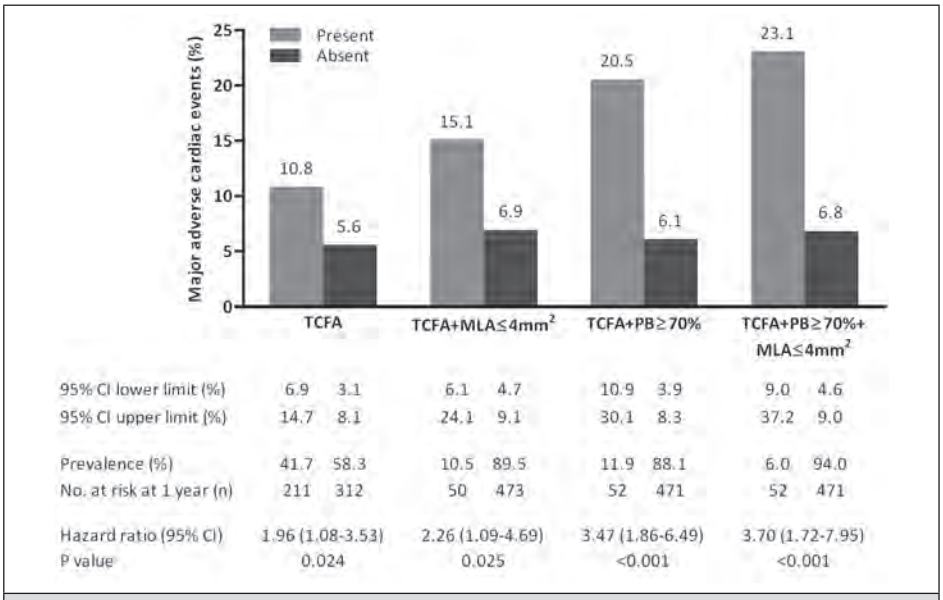

Figure 3. Cumulative Kaplan-Meier incidence estimates of major adverse cardiac events
Percentages are 1-year cumulative Kaplan-Meier incidence estimates. Hazard ratios are estimated using univariate Cox proportional hazards regression analysis. P values are obtained with log-rank test.
MLA, minimal luminal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

TCFA lesions seemed to carry higher risk than non-calcified TCFA lesions, although the 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

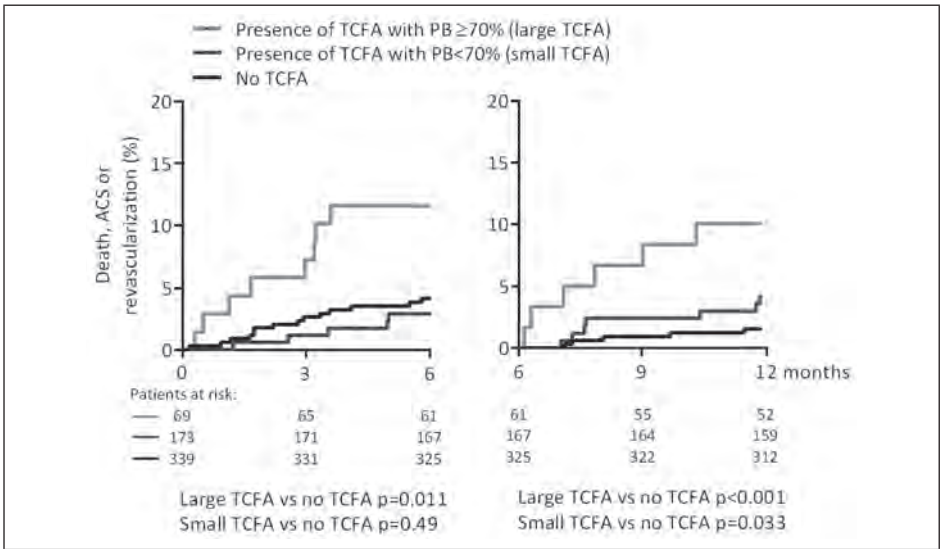

Figure 4. Associations with of short-term and long-term major adverse cardiac events
P values are obtained with log-rank test. Overall P value 0-6 months is 0.009; overall P value 6-12 months is 0.002.
PB, plaque burden; TCFA, thin-cap fibroatheroma.

when patients had a TCFA lesion with a minimal luminal area of 4.0 mm^2 or less, a plaque 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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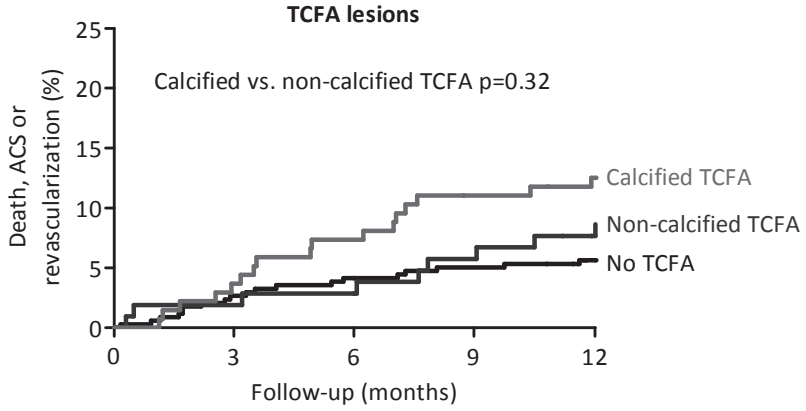
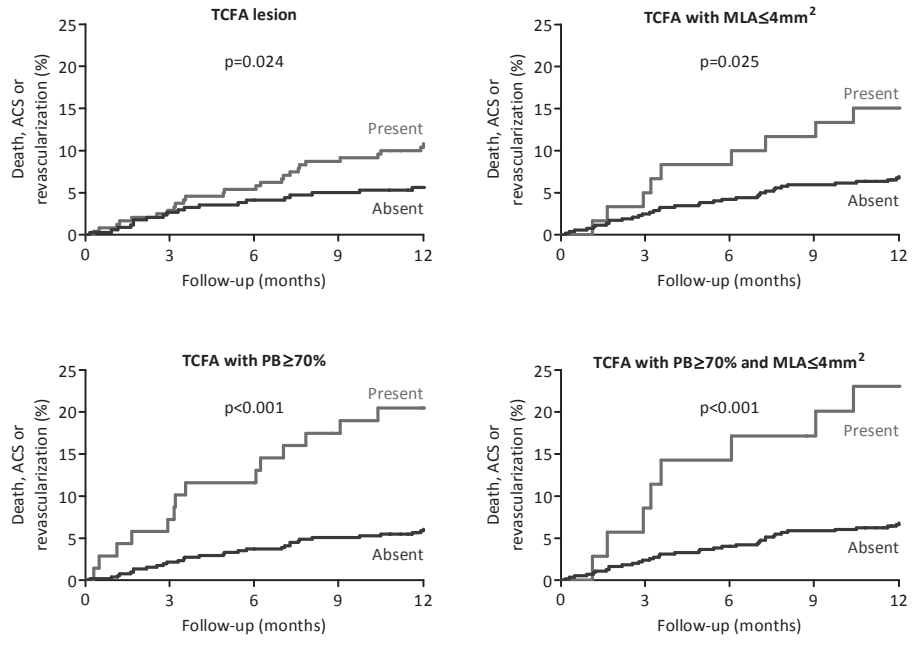
SUPPLEMENTAL FIGURES AND TABLES



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 discharges 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. 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
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.

Chapter 3

Near-infrared spectroscopy predicts cardiovascular outcome in patients with coronary artery disease

Oemrawsingh RM*, Cheng JM*, Garcia-Garcia HM, Van Geuns RJ, De Boer SPM, Simsek C, Kardys I, Lenzen MJ, Van Domburg RT, Regar E, Serruys PW, Akkerhuis KM, Boersma E.

* These authors contributed equally.

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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 ATHEROREMONIRS 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 allcause 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. Nonnormally 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).

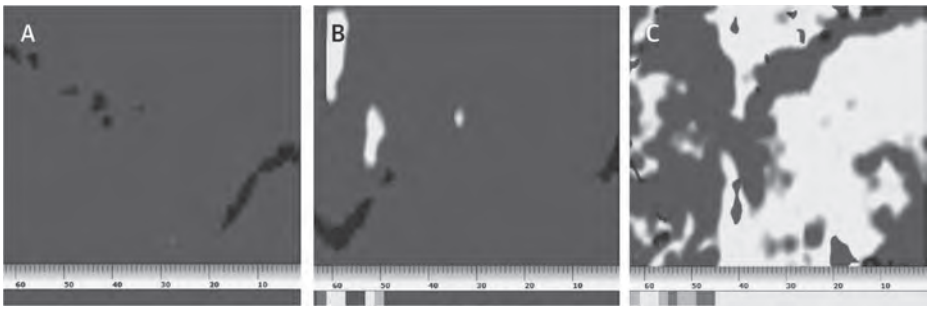

Figure 1. Intracoronary near-infrared spectroscopy displayed as a chemogram
The figures display the graphical result of coronary wall evaluation with near-infrared spectroscopy in 3 different patients. Spectral characteristics of lipid core– containing coronary plaques (LCP) are displayed along the length (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).
See also Appendix: Color figure 2.

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
Laboratory characteristics				
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) (Central Illustration). Cumulative event rates for other secondary endpoints—also exclusive of definite CLR events—also are shown in the Central Illustration.

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

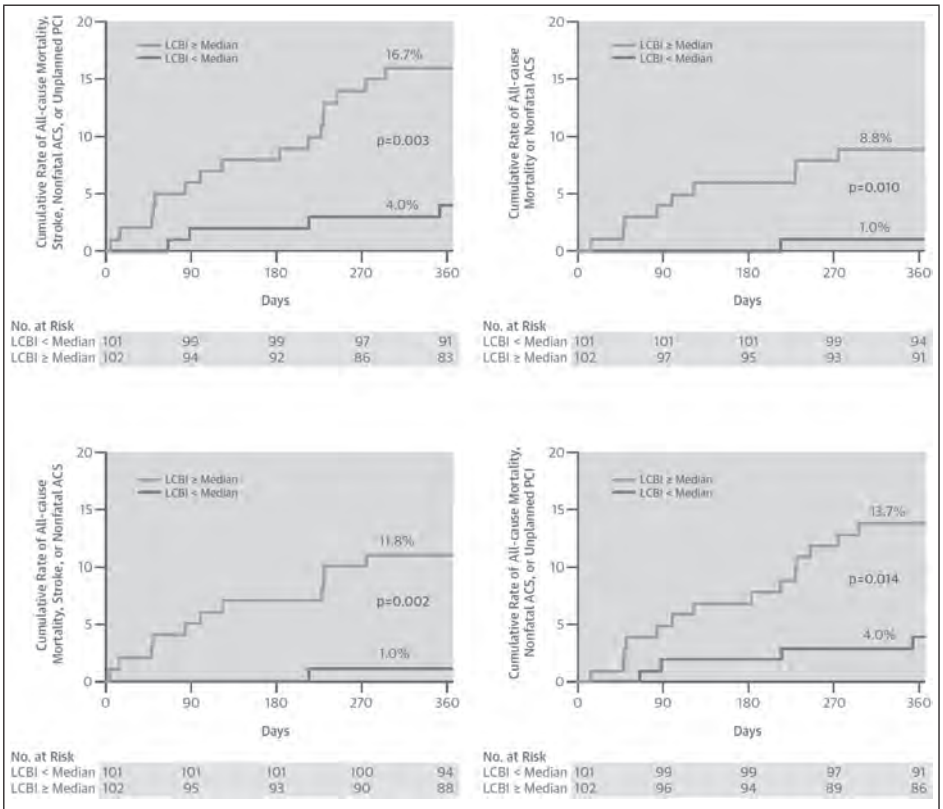

Figure 2. Time-to-event curves for the primary and pre-specified secondary composite endpoints exclusive of definite culprit lesion-related events

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 angiog-

raphy. 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 longterm 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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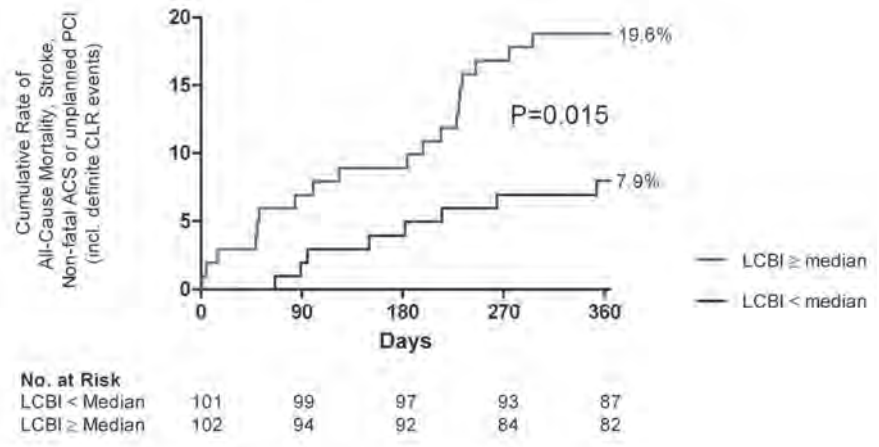
SUPPLEMENTAL TABLES AND FIGURES

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.

PART II

BIOMARKERS OF ATHEROSCLEROSIS

Chapter 4

High-sensitivity troponin-T in relation to coronary plaque characteristics in patients with stable coronary artery disease

Oemrawsingh RM*, Cheng JM*, Garcia-Garcia HM, Kardys I, Van Schaik RHN, Regar E, Van Geuns RJ, Serruys PW, Boersma E, Akkerhuis KM.

* These authors contributed equally.

Submitted for publication.

ABSTRACT

Aims: To assess the relationship between the extent and phenotype of coronary atherosclerosis, as assessed by radiofrequency intravascular ultrasound (IVUS) imaging, and circulating troponin levels in patients with stable coronary artery disease (CAD).

Methods and Results: Greyscale and virtual histology IVUS imaging of a non-culprit coronary artery was performed in 231 patients who underwent elective coronary angiography for stable CAD. High-sensitivity troponin T (hsTnT) was measured in blood that was drawn prior to the coronary angiography procedure. HsTnT was detectable (>3 pg/mL) in 212 patients (92%) and a concentration above 14 pg/mL (the 99th percentile of a healthy reference population, used as cut-off level to detect myocardial infarction) was observed in 19.5%. 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 IVUS virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesion (adjusted OR 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 (adjusted 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 (adjusted OR 3.36, 95%CI: 1.44-7.84, p=0.005).

Conclusion: 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) (1,2). In addition, Troponin elevations also have prognostic implications. Elevations of serum Troponin levels above the detection limit of conventional immunoassays (i.e. 0.01 µg per liter in case of Troponin T (TnT)), have been associated with increased mortality and recurrent ischemic events in patients admitted with an ACS (3,4).

Moreover, increased risk of mortality and occurrence of a first coronary event was also observed in subjects *without* clinical evidence of cardiovascular disease, but with circulating Troponin levels that were detectable by conventional assays (5). Furthermore, in ambulatory patients with established stable coronary artery disease (CAD) as enrolled in the Heart and Soul study, TnT was detectable in 6% of the study population when using a conventional TnT assay (6). 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 (7). In these 984 patients, higher hsTnT levels were, amongst others, associated with greater inducible ischemia, worse treadmill exercise capacity and left ventricular ejection fraction, but remained independently predictive of cardiovascular mortality, myocardial infarction (MI) and heart failure after adjustment for abnormalities in cardiac structure and function (7). 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 analysis of the PEACE trial (8) and in a study of stable CAD patients participating in an in-hospital rehabilitation program (9).

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. We therefore performed a prospective study in order 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 of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described previously (10). In brief, 581 patients with an indication for diagnostic coronary angiography (CAG) and/or percutaneous coronary intervention (PCI), as determined by their treating physician as part of routine clinical care, due to either stable angina pectoris (SAP) or an ACS were included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. Detailed inclusion and exclusion criteria have been listed elsewhere (10). ATHEROREMO-IVUS was enriched with eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib versus placebo (11). However, the latter cohort of additional IBIS-2 patients was not included in the present analysis in order to prevent treatment interaction from darapladib on Troponin levels, as was previously demonstrated (12). Furthermore, ACS patients were excluded from this analysis, 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. Similarly, patients with a history of heart failure and renal insufficiency, known confounders of Troponin elevation, 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 arterial sheath prior to the CAG procedure. The blood samples were transported to the clinical chemistry laboratory of the Erasmus MC for further processing and storage at a temperature of -80°C within 2 hours after blood collection. TnT was measured with both a conventional fourth generation assay and a high-sensitivity assay (Roche Diagnostics GmbH, Mannheim, Germany) 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 (13), which is commonly used as cut-off level to detect myocardial infarction. 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. 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 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 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. Both the IVUS gray-scale and virtual histology (IVUS-VH) 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). The extent and phenotype of each atherosclerotic plaque were assessed. 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 (14). 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 (15,16). 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 homoscedasticity). Segmental plaque volume was 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 (13), were also assessed with logistic regression. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, hypertension, smoking status, family history of CAD, history of MI, prior PCI, prior coronary artery bypass grafting, stroke and peripheral artery disease (PAD). Crude and adjusted odds ratios (OR) are presented with 95% confidence intervals (CI). 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. The prevalence of hypertension, hypercholesterolemia, diabetes mellitus and history of MI was 61%, 70%, 21% and 40%, respectively. 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 stable CAD. The 99th percentile in our patient population was 88.7 pg/mL (Table 1 and Figure 1).

Age was the only determinant of hsTnT concentration (adjusted (adj.) p<0.001) (Figure 2). Male gender (adj. p=0.39), diabetes (adj. p=0.96), hypertension (adj. p=0.64), hypercholesterolemia (adj. p=0.28), smoking status (adj. p=0.37), family history (adj. p=0.60), and history of MI (adj. p=0.57), PCI (adj. p=0.60), CABG (adj. p=0.46), CVA (adj. p=0.61), or PAD (adj. p=0.28), eGFR (adj. p=0.08) and the number of diseased coronary vessels on angiography (adj. p=0.71), did not determine hsTnT concentration.

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 3a). There was no significant association between segmental plaque burden and hsTnT (adjusted β= 0.92 % increase

Table 1. Baseline characteristics	
Patient characteristics	**N=231**
Age, years	63.6 ± 9.9
Male, n (%)	177 (76.6)
Diabetes Mellitus, n (%)	48 (20.8)
Hypertension, n (%)	141 (61.0)
Hypercholesterolemia, n (%)	162 (70.1)
Smoking, n (%)	42 (18.2)
Positive family history of CAD, n (%)	137 (59.3)
Previous MI, n (%)	93 (40.3)
Previous PCI, n (%)	116 (50.2)
Previous CABG, n (%)	7 (3.0)
Previous stroke, n (%)	13 (5.6)
Peripheral artery disease, n (%)	19 (8.2)
Glomerular Filtration Rate (ml/min)	101.5 [80.2-123.0]
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, n (%)	22 (9.5)
1-vessel disease, n (%)	116 (50.2)
2-vessel disease, n (%)	70 (30.3)
3-vessel disease, n (%)	23 (10.0)
PCI / stent implantation, n (%)	196 (84.8)
IVUS segment characteristics	
Imaged coronary artery	
Left anterior descending, n (%)	76 (33.9)
Left circumflex, n (%)	76 (32.9)
Right coronary artery, n (%)	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

in plaque burden per SD increase in natural log-transformed hsTnT, 95 % CI: -0.61-2.44, p=0.24).

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

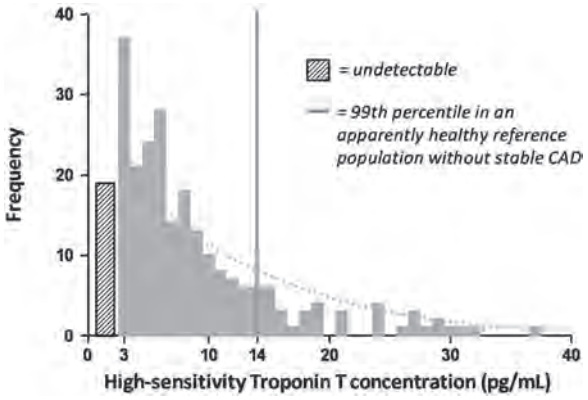


Figure 1. Distribution of high-sensitivity troponin T values in patients with stable coronary artery disease.

High-sensitivity troponin T was measured in 231 patients with stable coronary artery disease who underwent elective coronary angiography. The blood samples were drawn prior to the catheterisation procedure. Troponin T concentrations were undetectably low in 19 patients (8.2%). A concentration ≥14 pg/ml, which is based on the 99th percentile of an apparently healthy reference population (and therefore clinically used as a cut-off for myocardial infarction), was observed in 45 patients (19.5%). This might have clinical consequences as such patients may be misdiagnosed for having a non-ST-elevation myocardial infarction, e.g. in case of presentation at the emergency department with resting angina. The data points of three patients with concentrations of 82, 91 and 192 pg/ml respectively are not shown in this histogram.

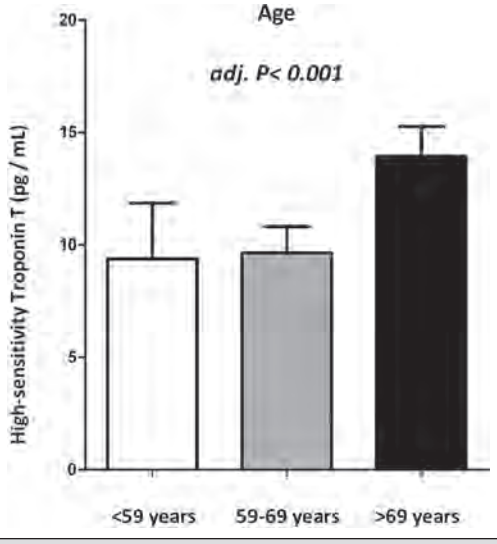


Figure 2. Age in relation to high-sensitivity troponin T concentration.

The study population was divided in age tertiles of 77 patients each. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, hypertension, smoking status, family history of coronary artery disease, history of myocarial infarction, prior percutaneous coronary intervention, prior coronary artery bypass grafting, prior stroke and peripheral artery disease. The histograms display the mean high-sensitivity troponin T concentration plus the standard error of the mean.

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 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 (%)***			
TCFA	86 (37.2)	64 (34.4)	22 (48.9)	0.024
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-TCFA with high lesional plaque volume was defined as VH-TCFA lesion with a plaque volume above the median of all lesions classified as VH-TCFA. Multivariable analyses accounted for confounding by age, gender, hypercholesterolemia, diabetes, hypertension, smoking status, family history of coronary artery disease, history of myocardial infarction, prior percutaneous coronary intervention, coronary artery by-pass grafting, stroke and peripheral artery disease.

HsTnT, high-sensitivity troponin T; VH-TCFA, virtual histology-derived thin-cap fibroatheroma; MLA, minimal luminal area

(adj. p=0.81) , fibro-fatty (adj. p=0.66), dense calcium (adj. p=0.37) and necrotic core (adj. p=0.80).

Association with lesion characteristics and morphology

With respect to lesion morphology, a VH-TCFA was observed in 86 (37%) patients (Table 2 and 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 a hsTnT concentration ≥ 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 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

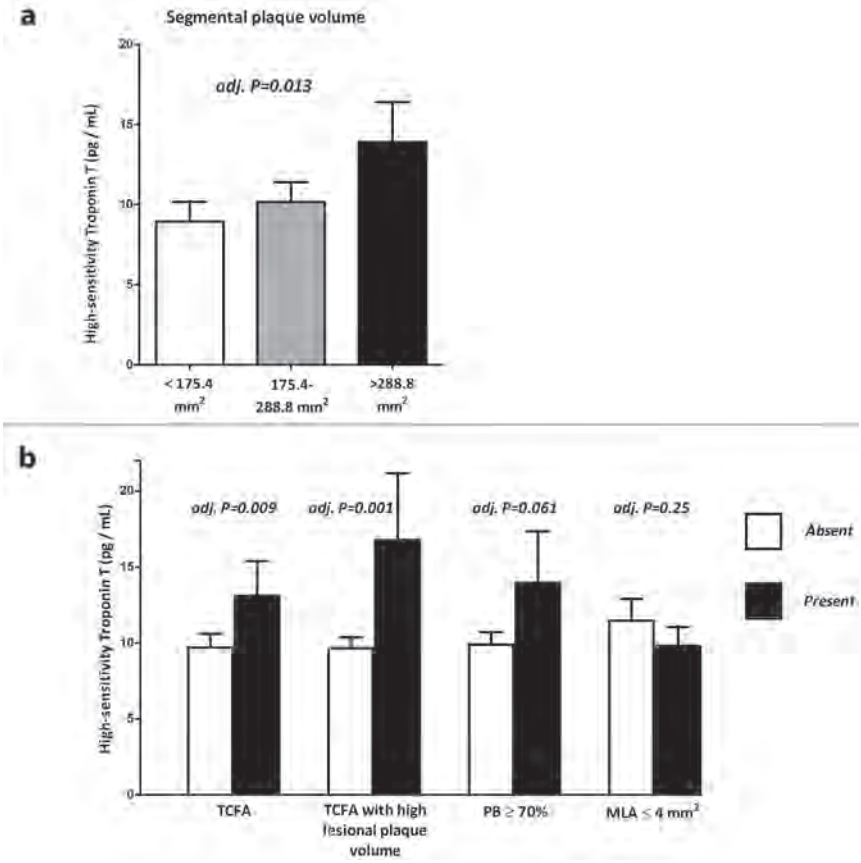


Figure 3. High-sensitivity troponin T concentration in relation to segmental plaque volume and lesion morphology.

Figure 3a: High-sensitivity troponin T per tertile of normalised plaque volume for the entire segment (median length 43.8mm, IQR: 33.9-56.0) as evaluated with greyscale intravascular ultrasound.

Figure 3b: High-sensitivity troponin T in relation to the presence of at least one high-risk lesion type per patient. Thin-cap fibroatheroma was assessed by intravascular ultrasound virtual histology. 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, 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.

Abbreviations: PB, plaque burden; MLA, minimal luminal area.

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,

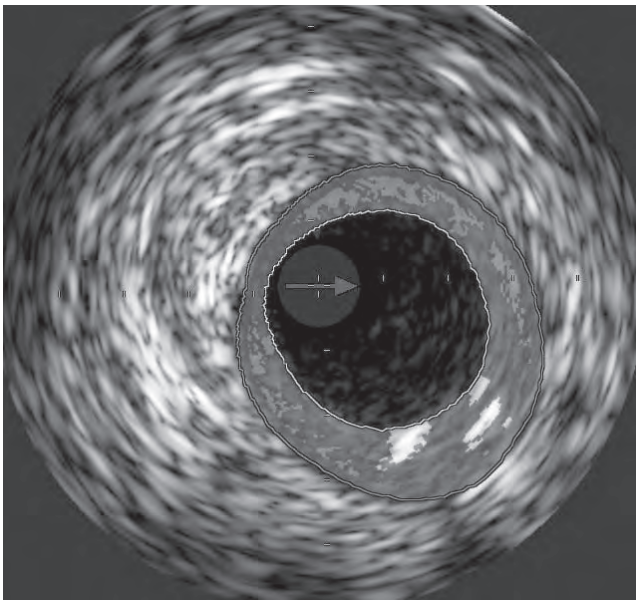


Figure 4. Thin-cap fibroatheroma as classified by radiofrequency intravascular ultrasonography
A lesion in the proximal right coronary artery of a 76–year old male ATHEROREMO-IVUS study participant with stable CAD and a history of hypertension, hypercholesterolemia and peripheral artery disease. This lesion (here only represented by one frame) was classified as thin-cap fibroatheroma with IVUS virtual histology. The tissue types are distinguished by colors: green=fibrous, yellow= fibro-fatty, white=dense calcium and red=necrotic core.
See also Appendix: Color figure 3.

95 % CI: 0.99-1.90, p=0.059) (Table 2 and Figure 3b). 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 prospective observational 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, cor-

responds to the results found in the Heart and Soul study (7). This expansion of serum TnT detection has led to more elaborate risk prediction for the occurrence of MACE, as previously demonstrated (7–9,17). 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 (8). 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 (18,19). 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 (18). More recently, similar conclusions were drawn with respect to the complete ATHEROREMO-IVUS study population comprising both stable CAD and ACS patients. 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 (20). Thus, both TnT elevation and high-risk intracoronary lesion phenotypes as assessed by (VH-) 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 (21,22). Cardiac computed tomography, however, does not allow for such extensive plaque phenotyping as IVUS-VH. 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 el-

evated 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 (23). Such plaque erosion or rupture has shown to be the major cause of (fatal) acute MI, but not every plaque ersion 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 atherotrombotic plaque events compared to an annual incidence of acute MI of approximately 5% (24). 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 asymtomatic 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% (17). 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 (21,22). More recently, a post-hoc analysis of the SATURN trial has demonstrated that a large plaque volume as assessed with grey-scale IVUS, in a non-culprit segment without significant stenosis, is associated with increased risk of MACE (25). 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 (23,24,26,27).

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. 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 (21,22). It indirectly also supports the hypothesis that not ischemia due to luminal narrowing, but rather plaque rupture and erosion leading to intracoronary thrombosis and microembolisation is the pathophysiological mechanism behind the increased circulating TnT levels.

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.

However, there are also 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. In our study, IVUS imaging took place of a prespecified 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 (20). 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 (25). 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.

In conclusion, segmental plaque volume and VH-TCFAs as assessed with intracoronary IVUS in a non-culprit coronary artery are associated with higher circulating Troponin concentrations in a broad population of patients with stable CAD. Subclinical plaque rupture or erosion and subsequent intracoronary thrombosis and distal embolization may be hypothesized as the potential mechanism of action with respect to Troponin elevation and its relation with adverse outcome.

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Chapter 5

Relation of C-reactive protein to coronary plaque characteristics on grayscale, radiofrequency intravascular ultrasound, and cardiovascular outcome

Cheng JM*, Oemrawsingh RM*, Garcia-Garcia HM, Akkerhuis KM, Kardys I, De Boer SP, Langstraat JS, Regar E, Van Geuns RJ, Serruys PW, Boersma E.

* These authors contributed equally.

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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,

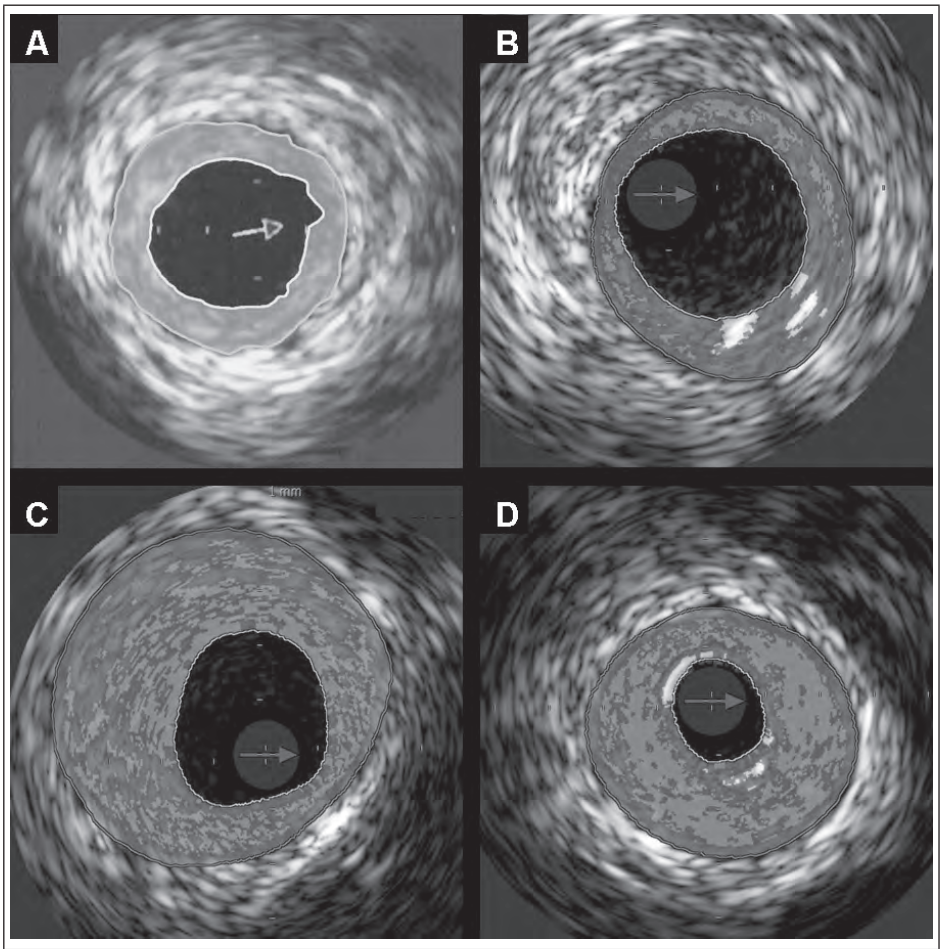

Figure 1. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound

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.

See also Appendix: Color figure 3.

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 endpoint 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.

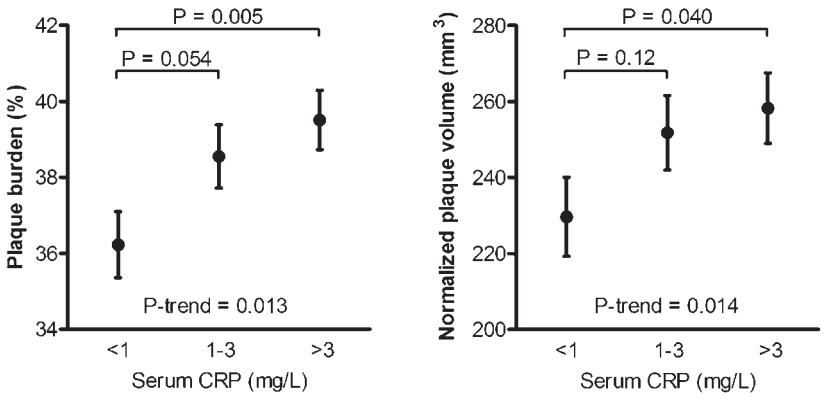

Figure 2. Association of CRP with coronary plaque burden and plaque volume of imaged coronary segment
CRP, C-reactive protein.

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

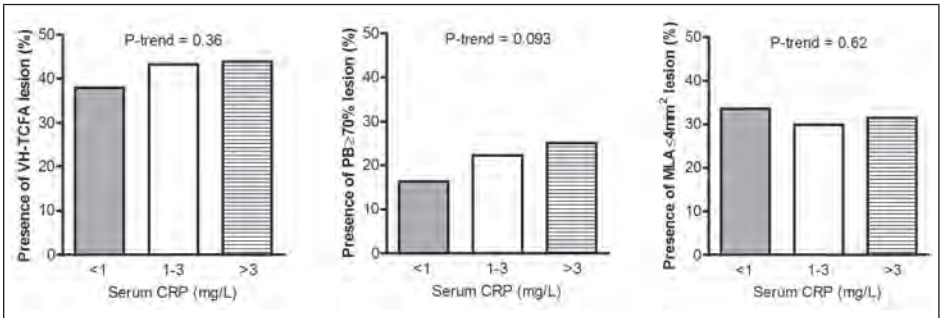

Figure 3. Association with presence of high risk coronary lesions
CRP, C-reactive protein; MLA, minimal luminal area; PB, plaque burden; VH-TCFA, virtual histology-derived thin-cap fibroatheroma.

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.

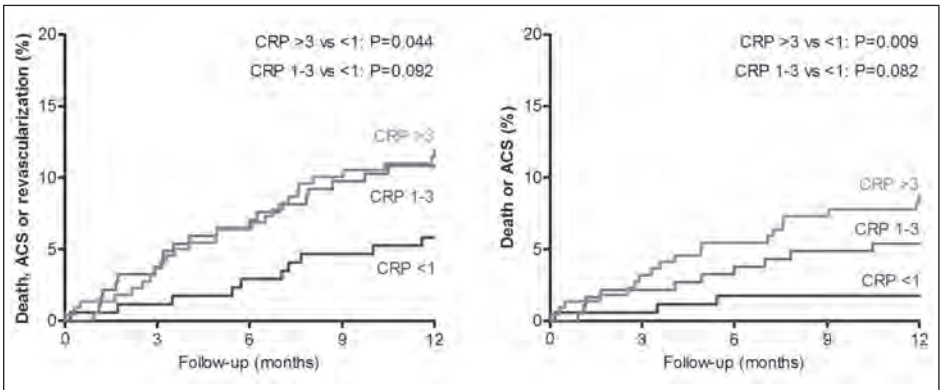

Figure 4. Prediction of cardiovascular outcome
ACS, acute coronary syndrome; CRP, C-reactive protein.

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.

Chapter 6

Circulating cytokines in relation to the extent and composition of coronary atherosclerosis

Battes LC*, Cheng JM*, Oemrawsingh RM, Boersma E, Garcia-Garcia HM, De Boer SP, Buljubasic N, Mieghem NA, Regar E, Geuns RJ, Serruys PW, Akkerhuis KM, Kardys I.

* These authors contributed equally.

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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. **Baseline characteristics** (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.

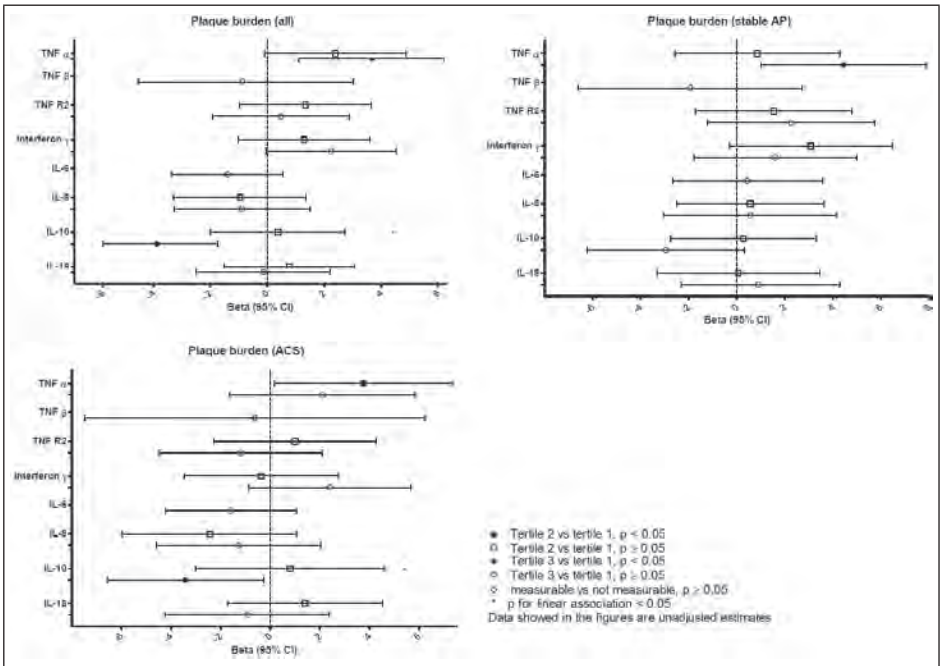

Figure 1. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in all patients, patients with stable AP and patients with ACS.

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.

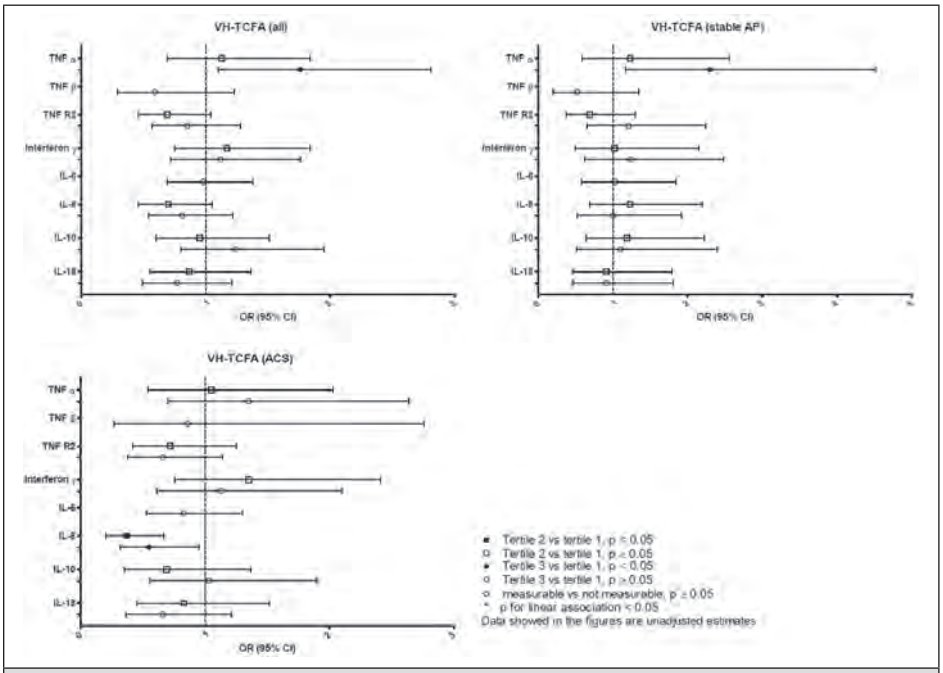

Figure 2. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in all patients, patients with stable AP and patients with ACS.

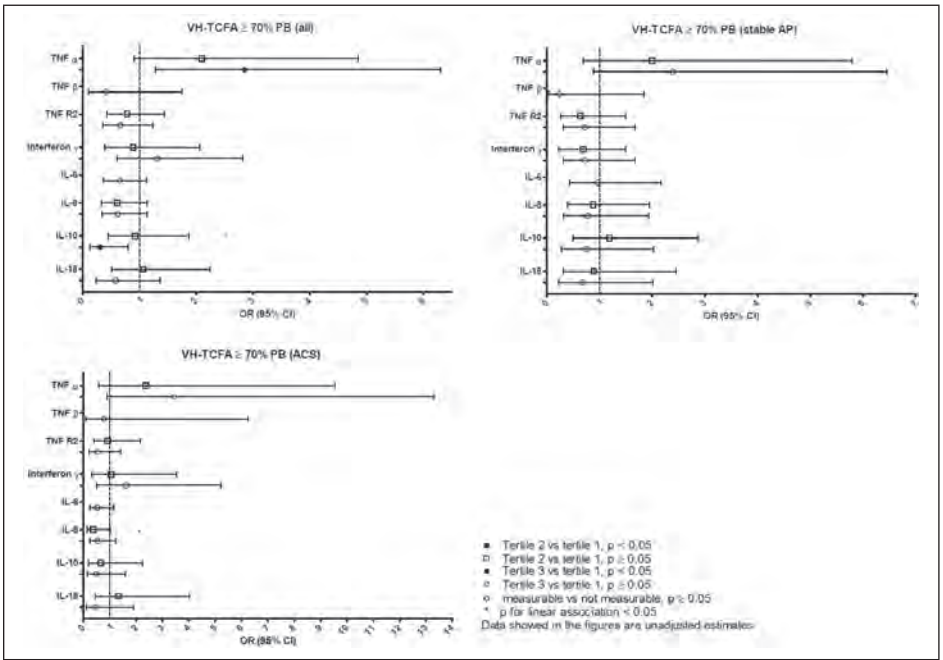

Figure 3. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in all patients, patients with stable AP and patients with ACS.

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

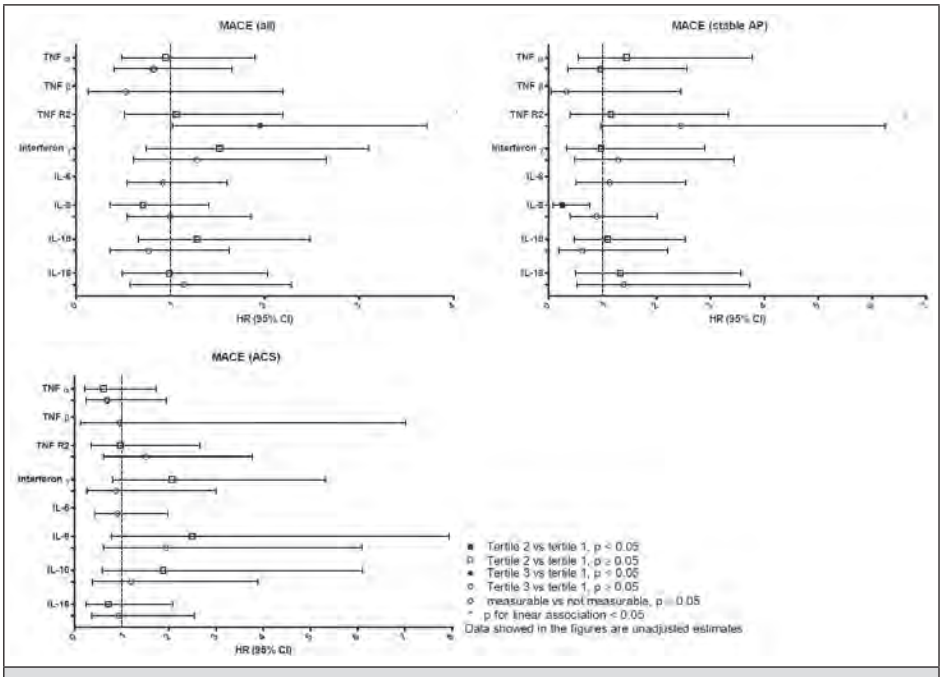

Figure 4. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in all patients, patients with stable AP and patients with ACS.

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.

Chapter 7

Circulating acute phase proteins in relation to extent and composition of coronary atherosclerosis and cardiovascular outcome

Battes LC, Akkerhuis KM, Cheng JM, Garcia-Garcia HM, Oemrawsingh RM, De Boer SPM, Regar E, Van Geuns RJ, Serruys PW, Boersma E, Kardys I.

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ABSTRACT

Introduction: Inflammation is characterized by increased plasma concentrations of acute phase proteins (APPs). We examined whether Alpha-1-Antitrypsin (AAT), Alpha-2-Macroglobulin (α2M), Complement C3 (C3), ferritin, haptoglobin, and Plasminogen Activator Inhibitor 1 (PAI-1) are associated with cardiovascular outcome, as well as with the extent and composition of coronary atherosclerosis as determined by intravascular ultrasound (IVUS) virtual histology (VH).

Methods: Between November 2008 and January 2011, IVUS(-VH) imaging of a non-culprit coronary artery was performed in 581 patients from the ATHEROREMO-IVUS study, who underwent coronary angiography for acute coronary syndrome (ACS) (n=318) or stable angina pectoris (SAP) (n=263). Coronary plaque volume was measured. Composition of atherosclerosis was characterized into 4 different types: fibrous, fibro-fatty, dense calcium and necrotic core. VH-derived thin-cap fibroatheroma (VH-TCFA) lesions were assessed. Major adverse cardiac events (MACE; all-cause mortality, ACS or unplanned coronary revascularization) were assessed during 1-year follow-up. We applied linear, logistic and Cox regression.

Results: Mean age was 61.5 ± 11.4 years and 75.4% were men. Higher ferritin was associated with higher coronary plaque volume (beta (β) [95%CI]: 0.19 [0.07-0.31] percent atheroma volume, for the highest vs the lowest tertile of ferritin; p for linear association =0.013. Higher PAI-1 was associated with higher rates of all-cause mortality or ACS (hazard ratio (HR) [95%CI]: 2.98 [1.10-8.06], for the highest vs the lowest tertile of PAI-1. No clear-cut associations could be demonstrated between APPs and composition of coronary atherosclerosis or IVUS-VH derived TCFA lesions.

Conclusions: Higher circulating ferritin was associated with higher coronary plaque volume, and higher PAI-1 was associated with higher incidence of all-cause mortality or ACS during 1-year follow-up. None of the APPs examined in this study displayed consistent associations with composition of atherosclerosis or plaque vulnerability.

INTRODUCTION

Chronic inflammation of the arterial wall plays an important role in the development of atherosclerosis, it regulates aspects of plaque biology that trigger the thrombotic complications of atherosclerosis [1]. Inflammation is commonly characterized by increased plasma concentrations of acute phase proteins (APPs). Several studies have demonstrated the ability of the APP C-reactive protein (CRP) to predict adverse coronary events in patients with stable and unstable coronary artery disease (CAD)[2]. In order to further explore the nature of the association of CRP with coronary atherosclerosis, we have previously examined its relation with intravascular ultrasound (IVUS) virtual histology (VH) derived measures of coronary atherosclerosis[3]. The results showed that, higher CRP levels were associated with a higher coronary plaque burden, but that they were not associated with plaque vulnerability, which was defined as the presence of IVUS-VH- derived thin-cap fibroatheroma (VH-TCFA) lesions.The relation between other APPs and cardiovascular disease has generally been examined to a much smaller extent, and in particular studies on APPs in relation to an in-vivo assessment of the extent and composition of coronary atherosclerosis are lacking [4, 5].

APP's including Alpha-1-Antitrypsin (AAT), Alpha-2-Macroglobulin (α2M), Complement C3 (C3), ferritin, haptoglobin, and Plasminogen Activator Inhibitor 1 (PAI-1), are produced by the liver in response to circulating cytokines[6] . APPs contribute to the restoration of homeostasis by neutralizing inflammatory agents, help to minimize the extent of local tissue damage and participate in tissue repair and regeneration[6]. Circulating levels of APPs may potentially be useful for risk stratification of patients with known CAD, and studies on their relation with the extent and composition of coronary atherosclerosis may provide further pathophysiological insights with regard to the mechanisms of progression and destabilization of atherosclerotic plaques.

Therefore, the purpose of this study is to examine the associations of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with the extent and composition of coronary atherosclerosis as determined in-vivo by IVUS-VH, in patients undergoing coronary angiography. Furthermore, the prognostic value of the APPs for major adverse cardiac outcome during 1 year follow-up in these patients is investigated.

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 [7]. 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 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 the Erasmus MC for further processing and storage at a temperature of -80°C within two hours after blood collection. CRP was measured in the clinical laboratory of the Erasmus MC 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). 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 AAT, α2M, C3, ferritin, haptoglobin, and PAI-1, were measured using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA). While ferritin, haptoglobin, and PAI-1 were determined in the full cohort of 570 patients, AAT, α2M, and C3, 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.

Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit coronary artery without significant coronary disease requiring balloon angioplasty or stent treatment. 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 Vulcano 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 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 volume was defined as the percent of the volume of the external elastic membrane occupied by atheroma, i.e. percent atheroma volume[8]. Plaque volume was normalized for the length of the imaged segment. 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 more than 40% in at least 3 consecutive frames. Using IVUS-VH, the composition of the atherosclerotic plaque was characterized into 4 different types: fibrous, fibro-fatty, dense calcium and necrotic core [9]. A IVUS-VH-derived thin-cap fibroatheroma (VH-TCFA) lesion was defined as a lesion with presence of > 10% confluent necrotic core in direct contact with the lumen.

Clinical study endpoints

In this study, follow-up lasted up to 1 year after 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. The primary endpoint was the occurrence of MACE, defined as the composite of all-cause mortality, ACS or unplanned coronary revascularization. The secondary endpoint was the composite of all-cause mortality or ACS. 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.[10, 11] Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). 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 biomarker levels are presented as medians (with 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 or were transformed by using the square root.

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 (transformed or categorized when appropriate) and, consecutively, plaque volume and plaque burden in the imaged coronary segment as the dependent variable. The results are presented as βs (per unit increase in transformed biomarker concentration or per category of biomarker concentration) with 95% confidence intervals (95% CIs).

Subsequently, we examined the associations between biomarker concentrations and 4 types of atherosclerosis composition (fibrous, fibrofatty, necrotic core, and dense calcium), each expressed in percentages. The results are again presented as βs. We also examined the associations between biomarker concentrations and the presence of VH-TCFA lesions by using logistic regression analyses with biomarker concentrations as the independent variable. The results are presented as odds ratios (ORs) per unit increase in transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

Moreover, we examined associations of biomarker concentrations with MACE and with the composite of all-cause mortality or ACS, 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. 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.

All above-described analyses were performed univariably. Subsequently, we adjusted for age, gender, indication for coronary angiography, diabetes, hypertension and CRP. Additionally, to further examine possible effect modification by indication for baseline coronary angiography, we repeated the analyses separately in patients with acute coronary syndrome and in patients with stable angina pectoris.

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% were men. Coronary angiography or PCI was performed for several indications:

Table 1. Baseline characteristics

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Patient characteristics			
Age, years, mean±standard deviation	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)
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]
Alpha-1-Antitrypsin (mg/mL)*, median (IQR)	1.40 [1.20-1.70]	1.40 [1.20-1.70]	1.40 [1.20-1.60]
Alpha-2-Macroglobulin (mg/mL)*, median (IQR)	1.50 [1.40-1.80]	1.50 [1.30-1.80]	1.60 [1.40-1.80]
Complement C3 (mg/mL)*, median (IQR)	0.90 [0.78-1.10]	0.90 [0.78-1.10]	0.92 [0.79-1.00]
Ferritin (mg/mL)#, median (IQR)	173.00 [94.50-282.50]	191.00 [102.25-319.00]	144.00 [82.00-242.50]
Haptoglobin (mg/mL)#, median (IQR)	1.40 [0.91-2.01]	1.50 [0.98-2.20]	1.26 [0.85-1.90]
Plasminogen Activator Inhibitor 1 (ng/mL)+, median (IQR)	35.00 [23.00-53.00]	38.00 [27.00-61.50]	31.00 [21.00-46.00]

AAT, α2M, and C3: total n= 473, ACS n=309, SAP n= 261

*Measurable in all 473 patients

+Measurable in all 570 patients

#Measurable in >99% of 570 patients, too low to detect in <1%

159 (28%) patients had an acute myocardial infarction, 150 (26%) patients had unstable angina pectoris and 261 (46%) patients had stable angina pectoris. The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm. C3 was the only biomarker with a normal distribution. AAT, α2M, ferritin, haptoglobin and PAI-1 concentrations were not normally distributed; haptoglobin was square root-transformed for further analyses, and the remaining biomarkers were ln-tranformed.

Biomarkers and extent of atherosclerosis

The results of the analyses for (ln-transformed) normalized plaque volume normalized for the length of the segment are shown in table 2. Higher ferritin levels were associated with higher coronary plaque volume (β [95%CI]: 0.19 [0.07-0.31], for the highest vs the lowest tertile of ferritin, and β [95%CI]: 0.14 [0.02-0.27], for the middle vs the lowest tertile of ferritin; p for linear association =0.01). After multivariable adjustment, only the association for the highest vs the lowest tertile of ferritin persisted (Table 2). In a post-hoc analysis, we performed the multivariable adjustment without adding CRP to the model (thus, only adjusting for age, gender, indication, diabetes and hypertension). The association was independent of these clinical covariates (β [95%CI]: 0.17 [0.05-0.30], for the highest vs the lowest tertile of ferritin, and β [95%CI]: 0.13 [0.006-0.25], for the middle vs the lowest tertile of ferritin; p for linear association =0.045) No associations were present between any of the other biomarkers and coronary plaque volume.

Associations with plaque burden are depicted in Supplementary table 1. Higher ferritin was associated with higher plaque burden (β [95%CI]: 1.22 [0.07-2.38], for the highest vs the lowest tertile of ferritin). However, p for linear association was not statistically significant (p=0.24).

Biomarkers and composition of atherosclerosis

The results of the analyses for composition of atherosclerosis are shown in Figure 1 and Supplementary Table 2-a, 2-b,2-c, and 2-d. Higher α2M levels were associated with a lower percentage of fibrous tissue (β [95%CI]: -4.60 [-7.10 - -2.10], for the highest vs the lowest tertile of α2M, and β [95%CI]: -2.65 [-5.02- -0.28], for the middle vs the lowest tertile of α2M; p for linear association =0.005). Furthermore, a higher α2M level was associated with a higher percentage of dense calcium tissue (β [95%CI]: 0.49 [0.23-0.375], for the highest vs the lowest tertile of α2M, and β [95%CI]: 0.30 [0.05-0.55], for the middle vs the lowest tertile of α2M; p for linear association =0.003). After multivariable adjustment, the associations for the highest tertiles of α2M remained significant, but the trends lost significance (Supplementary table 2-a and 2-d). No associations were present between α2M and the remaining atherosclerosis components.

Higher ferritin levels were associated with a higher percentage of fibrofatty tissue (β [95%CI]: 0.25 [0.05-0.46], for the highest vs the lowest tertile of ferritin; p for linear

Table 2. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with segment plaque volume

	Unadjusted model		Multivariable model*	
Segmental plaque volume	beta (95%CI)	P	beta (95%CI)	P
AAT				
Tertile 1	(reference)		(reference)	
Tertile 2	0.11 (-0.01-0.23)	0.08	0.08 (-0.04-0.21)	0.19
Tertile 3	0.09 (-0.04-0.22)	0.19	0.10 (-0.04-0.24)	0.15
Ln (AAT)	0.07 (-0.13-0.27)	0.50	0.03 (-0.19-0.24)	0.82
α2M				
Tertile 1	(reference)		(reference)	
Tertile 2	0.01 (-0.11-0.14)	0.83	-0.003 (-0.13-0.12)	0.97
Tertile 3	0.06 (-0.07-0.18)	0.40	0.08 (-0.05-0.22)	0.23
Ln (α2M)	-0.10 (-0.35-0.14)	0.40	-0.09 (-0.35-0.16)	0.47
C3				
Tertile 1	(reference)		(reference)	
Tertile 2	0.02 (-0.11-0.15)	0.76	0.00 (-0.13-0.13)	1.00
Tertile 3	-0.05 (-0.18-0.07)	0.39	-0.03 (-0.16-0.10)	0.66
C3	-0.16 (-0.41-0.10)	0.22	-0.15 (-0.41-0.12)	0.27
Ferritin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.14 (0.02-0.27)	0.023	0.12 (-0.01-0.24)	0.061
Tertile 3	0.19 (0.07-0.31)	0.003	0.16 (0.03-0.29)	0.014
Ln (Ferritin)	0.07 (0.02-0.13)	0.013	0.05 (-0.01-0.11)	0.077
Haptoglobin				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.04 (-0.16-0.09)	0.58	-0.02 (-0.15-0.11)	0.76
Tertile 3	-0.004 (-0.13-0.12)	0.95	0.003 (-0.12-0.13)	0.95
square root (haptoglobin)	-0.003 (-0.13-0.13)	0.97	-0.01 (-0.15-0.13)	0.89
PAI-1				
Tertile 1	(reference)		(reference)	
Tertile 2	0.09 (-0.04-0.21)	0.17	0.09 (-0.03-0.21)	0.15
Tertile 3	-0.03 (-0.15-0.10)	0.65	0.03 (-0.10-0.15)	0.67
Ln (PAI-1)	-0.02 (-0.10-0.06)	0.70	0.03 (-0.06-0.11)	0.55

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

association=0.015) (Figure 1 and Supplementary table 2-b). The trend disappeared after multivariable adjustment. Ferritin was not associated with other VH-IVUS-defined components.

Remaining biomarkers did not display any associations with the 4 components of atherosclerosis (Supplementary table 2-a, 2-b, 2-c, and 2-d.) Furthermore, associations

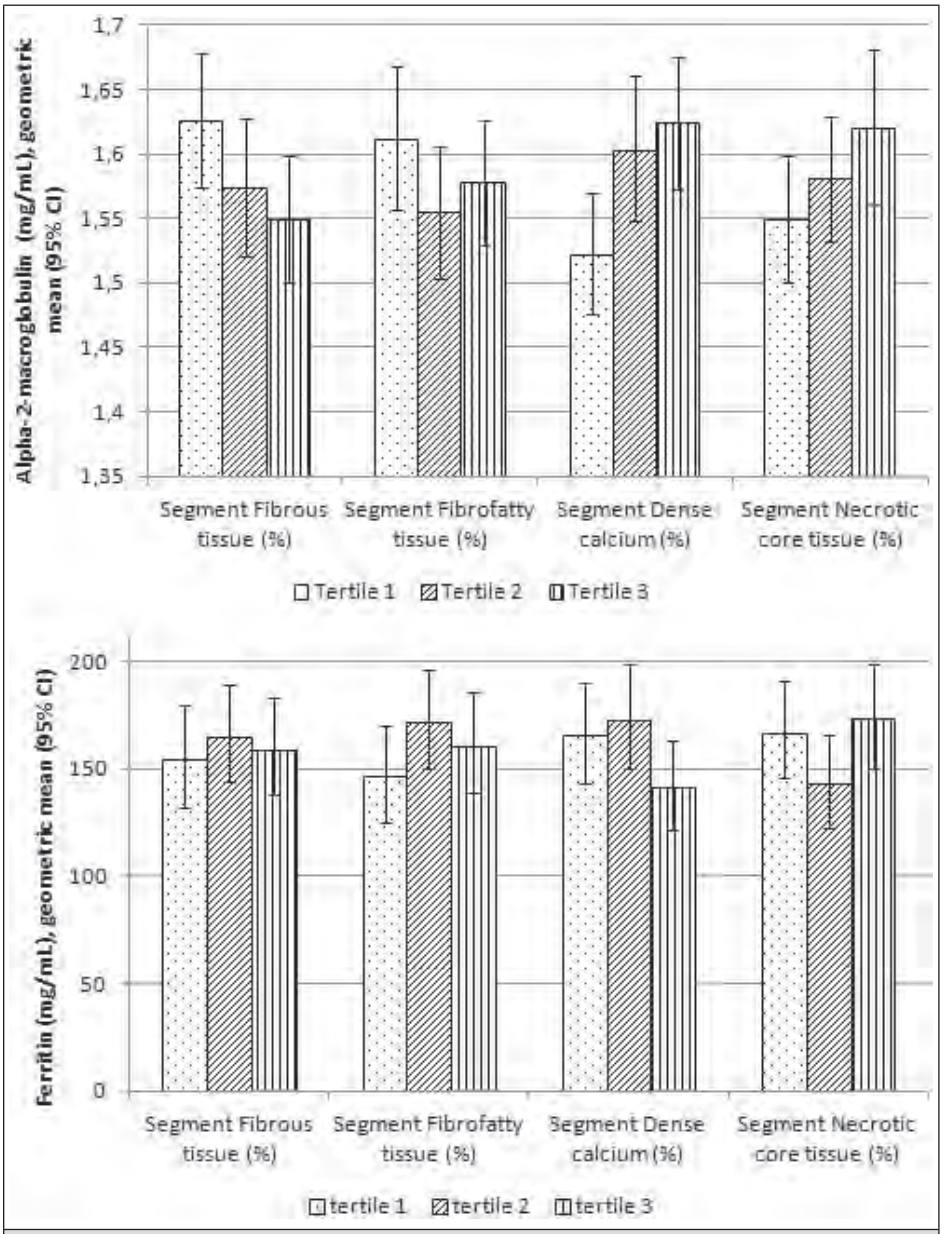

Figure 1. Univariable associations of Alpha-2-Macroglobulin and ferritin with composition of atherosclerosis

with individual components of atherosclerosis were not reflected by associations with IVUS-VH-derived TCFA: none of the biomarkers were associated with IVUS-VH derived TCFA (Table 3).

Table 3. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with presence of IVUS-VH derived TCFA lesions

	Unadjusted model		Multivariable model*	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
AAT				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.35 (0.85-2.15)	0.20	1.24 (0.78-1.99)	0.37
Tertile 3	1.19 (0.75-1.91)	0.46	1.09 (0.66-1.77)	0.74
LN (AAT)	1.13 (0.55-2.33)	0.74	0.94 (0.42-2.12)	0.89
α2M				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.09 (0.71-1.70)	0.69	1.16 (0.74-1.81)	0.53
Tertile 3	1.07 (0.67-1.69)	0.79	1.16 (0.71-1.89)	0.55
LN (α2M)	1.00 (0.41-2.41)	0.99	1.22 (0.47-3.13)	0.68
C3				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.05 (0.67-1.66)	0.83	1.02 (0.64-1.63)	0.92
Tertile 3	0.99 (0.63-1.56)	0.97	0.95 (0.60-1.52)	0.84
C3	0.72 (0.29-1.79)	0.48	0.58 (0.22-1.56)	0.28
Ferritin				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.09 (0.72-1.63)	0.69	1.07 (0.70-1.62)	0.77
Tertile 3	1.04 (0.69-1.57)	0.85	0.96 (0.63-1.48)	0.87
LN (Ferritin)	1.03 (0.86-1.25)	0.73	1.01 (0.83-1.23)	0.92
Haptoglobin				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.07 (0.71-1.60)	0.76	1.05 (0.69-1.58)	0.84
Tertile 3	0.81 (0.53-1.22)	0.31	0.75 (0.48-1.16)	0.19
Square root (haptoglobin)	0.81 (0.52-1.25)	0.34	0.72 (0.45-1.18)	0.19
PAI-1				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.05 (0.70-1.58)	0.81	0.98 (0.65-1.49)	0.93
Tertile 3	1.00 (0.66-1.49)	0.98	0.94 (0.62-1.43)	0.77
LN (PAI-1)	1.05 (0.80-1.37)	0.73	1.01 (0.76-1.33)	0.96

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Biomarkers and outcome

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 experienced at least 1 MACE (%). No significant associations were found between the APPs

and MACE (hazard ratios for the occurrence of MACE are shown in Supplementary Table 3). A total of 30 cases of all-cause mortality or ACS occurred. Hazard ratios for the occurrence of the composite of all-cause mortality or ACS are shown in Table 4. Higher PAI-1

Table 4. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with combination mortality and ACS

All-cause mortality or ACS	Unadjusted model		Multivariable model*		Multivariable model#	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
AAT						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.37 (0.10-1.38)	0.14	0.18 (0.03-1.07)	0.059	0.30 (0.07-1.20)	0.089
Tertile 3	0.33 (0.08-1.267)	0.11	0.18 (0.03-0.97)	0.046	0.556 (0.13-2.36)	0.43
Ln (AAT)	0.31 (0.06-1.72)	0.18	0.30 (0.06-1.59)	0.16	0.64 (0.07-5.66)	0.69
α2M						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.35 (0.52-3.51)	0.54	1.45 (0.54-3.91)	0.47	1.18 (0.32-4.41)	0.81
Tertile 3	1.34 (0.46-3.87)	0.59	1.35 (0.43-4.28)	0.61	1.44 (0.33-6.24)	0.63
Ln (α2M)	2.99 (0.38-23.62)	0.30	3.26 (0.35-30.86)	0.30	3.02 (0.14-66.71)	0.48
C3						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.99 (0.35-2.81)	0.99	1.12 (0.35-3.54)	0.85	0.73 (0.23-2.32)	0.59
Tertile 3	1.77 (0.60-5.23)	0.30	2.41 (0.51-11.32)	0.27	2.11 (0.68-6.57)	0.20
C3	1.60(0.11-23.40)	0.73	1.08 (0.02-61.46)	0.97	2.94 (0.18-48.21)	0.45
Ferritin						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.80 (0.30-2.10)	0.65	1.03 (0.35-2.98)	0.96	0.56 (0.20-1.57)	0.27
Tertile 3	1.54 (0.62-3.84)	0.36	2.21 (0.71-6.93)	0.17	1.34 (0.53-3.43)	0.54
Ln (Ferritin)	1.04 (0.71-1.53)	0.83	1.12 (0.74-1.70)	0.59	0.98 (0.64-1.49)	0.91
Haptoglobin						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.37 (0.14-1.00)	0.049	0.33 (0.12-0.94)	0.037	0.434 (0.16-1.20)	0.12
Tertile 3	0.49 (0.20-1.18)	0.11	0.40 (0.14-1.10)	0.074	0.89 (0.30-2.66)	0.84
square root (haptoglobin)	0.77 (0.31-1.87)	0.56	0.64 (0.23-1.76)	0.39	1.04 (0.40-2.76)	0.93
PAI-1						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	3.46 (1.30-9.22)	0.013	3.57 (1.32-9.66)	0.012	3.64 (1.27-10.43)	0.016
Tertile 3	2.98 (1.10-8.06)	0.032	4.12 (1.04-16.41)	0.045	3.51 (1.26-9.74)	0.016
Ln (PAI-1)	1.52 (0.94-2.46)	0.091	2.02 (0.99-4.09)	0.052	1.74 (1.01-2.99)	0.047

*adjusted for age, gender and indication for coronary angiography
#additionally adjusted for diabetes mellitus, hypertension and CRP

was associated with higher event rates (HR[95%CI]: 2.98 [1.10-8.06], for the highest vs the lowest tertile of PAI-1, and HR[95%CI]: 3.46 [1.30-9.22], for the middle vs the lowest tertile of PAI-1). After multivariable adjustment, associations remained significant. No associations were present between any of the other biomarkers and all-cause mortality or ACS.

Patients with ACS versus stable angina pectoris

To further investigate the possibility of effect modification by baseline indication for coronary angiography, we repeated all analyses separately in patients with ACS and patients with SAP. The association between ferritin and higher percentage of fibrofatty tissue was present in patients with ACS (β [95%CI]: 0.56 (0.27-0.86) for the highest vs the lowest tertile of ferritin, p for linear association < 0.001), but was not present in patients with SAP. Remaining estimates remained materially the same in both subgroups, although statistical significance disappeared on some occasions because of less power in the subgroups.

DISCUSSION

In this paper, we examined whether circulating APP concentrations are associated with the extent and composition of coronary atherosclerosis, as determined by (VH)-IVUS, in patients undergoing coronary angiography. We also investigated whether these APPs have prognostic value for clinical cardiovascular outcome. Higher concentrations of ferritin were associated with higher coronary plaque volume as well as a higher percentage of fibrofatty tissue in the coronary atherosclerotic plaque, the latter only in patients with acute coronary syndrome at baseline. Furthermore, higher concentrations of α2M were associated with lower percentage of fibrous tissue and higher percentage of dense calcium tissue in the coronary atherosclerotic plaque. In spite of these associations with individual components of atherosclerosis, none of the biomarkers were associated with presence of IVUS-VH derived TCFA lesions. Higher concentrations of PAI-1 were associated with the composite endpoint of all-cause mortality or ACS. No significant associations were found between the other APPs and the clinical endpoints.

Ferritin plays a fundamental role in the storage of intracellular iron. Consequently, this marker is widely used in diagnosing and monitoring of diseases associated with iron overload or iron deficiency[12]. Tran et al. [13] showed, in vivo, that serum ferritin concentration increases in response to the inflammatory cytokines interleukin-1 and TNF-α, suggesting that these cytokines upregulate ferritin and its secretion. Elevated serum concentrations of ferritin are seen in several inflammatory conditions like chronic kidney disease, acute infection, and malignancy[13]. Recently, Sung et al. [14] found a significant association between elevated levels of ferritin and the presence of coronary

artery calcium, a marker of preclinical atherosclerosis. This association was independent of cardiovascular risk factors, iron-binding capacity (transferrin), and low-grade inflammation. However, in general, clinical studies have given contradictory results regarding the ability of ferritin to predict cardiovascular events [15-18]. In our study, higher ferritin levels were clearly associated with higher coronary atherosclerotic plaque volume, but their association with higher plaque burden was less apparent. This seeming discrepancy 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 remodelling. Furthermore, higher ferritin levels were associated with higher percentage of fibrofatty tissue in coronary atherosclerotic plaque, the latter only in patients with acute coronary syndrome at baseline. However, no association was found with VH-TCFA lesions, nor with clinical cardiovascular events. Since serum concentrations of ferritin may vary in a wide range of conditions [19], the interpretation of ferritin level may be complicated and additional research is needed to confirm its potential association with extent of coronary atherosclerosis.

α2M is particularly known as an APP that can bind a large array of ligands and remove them from blood circulation to protect the body from wide disturbances [20]. Its primary function is the inhibition of fibrinolysis which, under normal physiological conditions, contributes to stabilization of vascular thrombus[21]. Furthermore, this protein is fundamental for enabling smooth muscle cells to attach, migrate and survive in fibrin [22], which suggests that this marker is involved in the mechanism of vascular stenosis. Clinical studies have shown that levels of α2M are significantly influenced by nephrotic syndrome, diabetes mellitus, and chronic liver disease [23]. Moreover, the cardiac isoform of A2macro seems to be useful in diagnosing cardiac events in patients with diabetes [24, 25] and in HIV patients [26, 27]. In the current study, we found a significant association of elevated α2M levels with presence of lower percentage of fibrous tissue and higher percentage dense calcium tissue in the atherosclerotic plaque. However, these associations with plaque composition could not be further translated into associations with number of VH-TCFA lesions.

PAI-1 was initially described as a protein that controls the plasminogen activation system, leading to inhibition of endogenous fibrinolysis and shifting the dynamic balance towards fibrin generation[28]. Circulating concentrations of PAI-1 are elevated in both the elderly [29] and in the presence of several clinical characteristics, including hypertriglyceridemia [29], obesity, insulin resistance, decreased immune responses, and increased inflammation[30]. Although PAI-1 may contribute to the development of the atherosclerotic plaque by stabilizing the fibrin matrix, this protein is also involved in, for example, vascular smooth muscle migration, and activation of some matrix metalloproteases (MMPs) that diminish plaque rupture[28, 31]. This phenomenon is also known as the 'PAI-1 paradox'[28]. Nevertheless, most epidemiological studies have showed that

PAI-1 is a risk factor for the development [32, 33] and recurrence of cardiovascular disease[34, 35]. Furthermore, a recent meta-analysis [36] has suggested that the presence of the 4G/5G gene polymorphism of PAI-1, resulting in increased PAI-1 levels, is associated with increased risk of MI. Our findings, demonstrating that higher concentrations of PAI-1 are associated with higher acute cardiac event rates, are in line with these prior studies.

Previous studies on AAT deficiency [37, 38] and complement factor C3 [39] and cardiovascular disease have rendered contradictory results. A recent study on common haptoglobin variants showed that these variants modify the inflammatory response to intraplaque hemorrhage and increase the risk of major cardiovascular events[40]. In particular, the hp2 allele of haptoglobin was associated with such events. In the current study, we could not demonstrate any associations of AAT, C3 and haptoglobin with coronary plaque characteristics or clinical events.

Some aspects of this study warrant consideration[7]. IVUS-VH imaging was performed in a prespecified single target segment of a single non-culprit coronary artery, based on the assumption that such a non-stenotic segment would adequately reflect coronary wall pathophysiology of the larger coronary tree[8]. 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, in fact, reflect larger coronary disease burden and is associated with subsequent cardiac events [41, 42]. Furthermore, it is important to note that IVUS is formally not capable of detecting TCFA lesions [7, 8, 43], because the spatial resolution of IVUS is insufficient for thin cap detection [8]. Nonetheless, a concept of IVUS-VH derived TCFAs 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 IVUS-VH frames [8]. Notably, we have recently demonstrated that such IVUS-VH derived TCFA lesions are strongly and independently predictive of the occurrence of MACE within the current study population[42].

CONCLUSIONS

In this population of patients undergoing coronary angiography for ACS or SAP, higher circulating ferritin was associated with higher coronary plaque volume, and higher PAI-1 was associated with higher incidence of all-cause mortality or ACS during 1-year follow-up. Of the APPs we investigated, no clear-cut associations could be demonstrated with composition of coronary atherosclerosis or with plaque vulnerability, as assessed by IVUS-VH. Further research, using various intravascular imaging modalities, is warranted to provide additional insights into potential mechanisms through which APPs may affect composition of atherosclerosis.

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SUPPLEMENTAL TABLES

Supplementary table 1. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with plaque burden

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
AAT (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.28 (-0.97-3.52)	0.26	0.58 (-1.64-2.81)	0.61
Tertile 3	0.82 (-0.39-2.03)	0.19	0.84 (-0.43-2.10)	0.20
LN (AAT)	1.47 (-2.23-5.17)	0.44	0.61 (-3.40-4.62)	0.77
α2M (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.37 (-1.90-2.63)	0.75	-0.34 (-2.59-1.90)	0.76
Tertile 3	0.71 (-0.46-1.88)	0.23	0.58 (-0.67-1.82)	0.36
LN (α2M)	-0.34 (-4.84-4.16)	0.88	-1.79 (-6.44-2.87)	0.45
C3 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	-0.66 (-3.03-1.72)	0.59	-0.51 (-2.87-1.84)	0.67
Tertile 3	-0.63 (-1.78-0.52)	0.28	-0.47 (-1.64-0.70)	0.43
C3	-3.48 (-8.13-1.16)	0.14	-3.37 (-8.17-1.44)	0.17
Ferritin (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.68 (-0.73-4.08)	0.17	1.71 (-0.66-4.08)	0.16
Tertile 3	1.22 (0.07-2.38)	0.038	1.10 (-0.10-2.30)	0.07
LN (Ferritin)	0.63 (-0.43-1.70)	0.24	0.39 (-0.70-1.48)	0.48
Haptoglobin (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	-1.03 (-3.44-1.39)	0.40	-0.74 (-3.11-1.62)	0.54
Tertile 3	0.06 (-1.10-1.22)	0.92	0.03 (-1.19-1.25)	0.96
SQRT (haptoglobin)	0.70 (-1.77-3.17)	0.58	0.52 (-2.14-3.19)	0.70
PAI-1 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.25 (-2.08-2.58)	0.83	0.25 (-2.09-2.58)	0.83
Tertile 3	-0.88 (-2.06-0.31)	0.15	-0.20 (-1.39-0.99)	0.74
LN (PAI-1)	-1.19 (-2.70-0.33)	0.13	-0.31 (-1.84-1.22)	0.69

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplementary table 2a. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with fibrous tissue

	Unadjusted model		Multivariable model*	
Fibrous tissue (%)	beta (95%CI)	P	beta (95%CI)	P
AAT				
Tertile 1	(reference)		(reference)	
Tertile 2	-1.08 (-3.42-1.26)	0.36	-1.02 (-3.39-1.35)	0.40
Tertile 3	-1.43 (-4.14-1.27)	0.30	-1.48 (-4.29-1.34)	0.30
Ln (AAT)	-2.34 (-6.35-1.68)	0.25	-3.53 (-7.90-0.84)	0.11
α2M				
Tertile 1	(reference)		(reference)	
Tertile 2	-2.65 (-5.02- -0.28)	0.029	-1.77 (-4.16-0.63)	0.15
Tertile 3	-4.60 (-7.10- -2.10)	<0.001	-3.36 (-6.02- -0.69)	0.014
Ln (α2M)	-6.97 (-11.88- -2.06)	0.005	-4.13 (-9.31-1.05)	0.12
C3				
Tertile 1	(reference)		(reference)	
Tertile 2	0.84 (-1.57-3.24)	0.50	0.47 (-1.94-2.88)	0.70
Tertile 3	-1.87 (-4.46-0.73)	0.16	-3.07 (-5.71- -0.43)	0.023
C3	-1.04 (-6.10-4.02)	0.69	-2.67 (-7.96-2.62)	0.32
Ferritin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.09 (-2.33-2.51)	0.94	-0.19 (-2.65-2.27)	0.88
Tertile 3	0.48 (01.99-2.94)	0.70	0.05 (-2.54-2.64)	0.97
Ln (Ferritin)	0.35 (-0.73-1.42)	0.52	0.24 (-0.88-1.36)	0.68
Haptoglobin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.71 (-1.74-3.17)	0.57	0.38 (-2.10-2.86)	0.76
Tertile 3	0.72 (-1.55-3.00)	0.53	0.39 (-1.99-2.77)	0.75
square root (haptoglobin)	0.05 (-2.45-2.55)	0.97	-0.47 (-3.21-2.26)	0.74
PAI-1				
Tertile 1	(reference)		(reference)	
Tertile 2	1.11 (-1.28-3.50)	0.36	0.49 (-1.94-2.92)	0.69
Tertile 3	0.02 (-2.38-2.41)	0.99	-1.21 (-3.66-1.23)	0.33
Ln (PAI-1)	0.01 (-1.53-1.55)	0.99	-0.79 (-2.36-0.78)	0.32

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplementary table 2b. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with fibrofatty tissue

	Unadjusted model		Multivariable model*	
Fibrofatty tissue (%)	beta (95%CI)	P	beta (95%CI)	P
AAT				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.09 (-0.289-0.11)	0.36	-0.05 (-0.24-0.15)	0.64
Tertile 3	-0.12 (-0.34-0.09)	0.26	-0.10 (-0.33-0.12)	0.37
Ln (AAT)	-0.24 (-0.56-0.09)	0.16	-0.22 (-0.57-0.13)	0.22
α2M				
Tertile 1	(reference)		(reference)	
Tertile 2	0.04 (-0.15-0.24)	0.65	0.02 (-0.18-0.22)	0.86
Tertile 3	-0.11 (-0.33-0.10)	0.29	-0.16 (-0.38-0.06)	0.15
Ln (α2M)	-0.22 (-0.62-0.18)	0.28	-0.31 (-0.73-0.11)	0.15
C3				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.02 (-0.23-0.18)	0.82	0.02 (-0.19-0.22)	0.88
Tertile 3	-0.17 (-0.38-0.04)	0.12	-0.14 (-0.35-0.07)	0.19
C3	-0.31 (-0.72-0.10)	0.14	-0.19 (-0.62-0.24)	0.38
Ferritin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.07 (-0.13-0.26)	0.50	0.06 (-0.13-0.26)	0.52
Tertile 3	0.25 (0.05-0.46)	0.015	0.21 (0.003-0.43)	0.047
Ln (Ferritin)	0.10 (0.01-0.19)	0.023	0.09 (-0.01-0.18)	0.068
Haptoglobin				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.10 (-0.31-0.11)	0.33	-0.08 (-0.28-0.13)	0.48
Tertile 3	-0.11 (-0.29-0.07)	0.22	-0.08 (-0.27-0.11)	0.41
square root (haptoglobin)	-0.19 (-0.39-0.02)	0.070	-0.17 (-0.39-0.06)	0.15
PAI-1				
Tertile 1	(reference)		(reference)	
Tertile 2	0.01 (-0.17-0.20)	0.89	0.07 (-0.11-0.26)	0.44
Tertile 3	0.06 (-0.15-0.26)	0.57	0.09 (-0.12-0.30)	0.39
Ln (PAI-1)	0.03 (-0.10-0.16)	0.66	0.07 (-0.05-0.20)	0.26

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplementary table 2c. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with necrotic core tissue

	Unadjusted model		Multivariable model*	
Necrotic core tissue (%)	beta (95%CI)	P	beta (95%CI)	P
AAT				
Tertile 1	(reference)		(reference)	
Tertile 2	1.19 (-0.40-2.77)	0.14	1.05 (-0.59-2.68)	0.21
Tertile 3	1.16 (-0.68-3.00)	0.21	1.09 (-0.86-3.03)	0.27
Ln (AAT)	2.14 (-0.60-4.87)	0.13	2.36 (-0.67-5.38)	0.13
α2M				
Tertile 1	(reference)		(reference)	
Tertile 2	0.22 (-1.42-1.85)	0.80	0.20 (-1.49-1.89)	0.82
Tertile 3	1.87 (0.10-3.64)	0.039	2.05 (0.16-3.94)	0.034
Ln (α2M)	2.49 (-0.88-5.86)	0.15	2.88 (-0.70-6.46)	0.12
C3				
Tertile 1	(reference)		(reference)	
Tertile 2	0.28 (-1.37-1.92)	0.74	0.22 (-1.46-1.90)	0.80
Tertile 3	1.47 (-0.24-3.18)	0.092	1.79 (0.003-3.58)	0.050
C3	1.657 (-1.792-5.106)	0.35	1.55 (-2.11-5.21)	0.41
Ferritin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.43 (-1.24-2.09)	0.62	0.41 (-1.30-2.11)	0.64
Tertile 3	-0.56 (-2.24-1.13)	0.52	-0.44 (-2.24-1.37)	0.64
Ln (Ferritin)	-0.33 (-1.08-0.41)	0.38	-0.37 (-1.15-0.42)	0.36
Haptoglobin				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.07 (-1.79-1.65)	0.94	-0.19 (-1.95-1.57)	0.83
Tertile 3	0.34 (-1.21-1.89)	0.67	0.22 (-1.43-1.86)	0.80
square root (haptoglobin)	1.14 (-0.59-2.87)	0.19	1.15 (-0.76-3.07)	0.24
PAI-1				
Tertile 1	(reference)		(reference)	
Tertile 2	0.21 (-1.43-1.84)	0.81	0.05 (01.61-1.71)	0.95
Tertile 3	0.07 (-1.59-1.74)	0.93	0.17 (-1.57-1.90)	0.85
Ln (PAI-1)	0.11 (-0.96-1.17)	0.84	-0.24 (-1.13-1.08)	0.97

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplementary table 2d. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with dense calcium tissue

	Unadjusted model		Multivariable model*	
Dense calcium tissue (%)	beta (95%CI)	P	beta (95%CI)	P
AAT				
Tertile 1	(reference)		(reference)	
Tertile 2	0.13 (-0.13-0.38)	0.32	0.10 (-0.16-0.35)	0.47
Tertile 3	0.08 (-0.21-0.37)	0.59	0.06 (-0.23-0.35)	0.68
Ln (AAT)	0.16 (-0.26-0.59)	0.46	0.24 (-0.22-0.70)	0.30
α2M				
Tertile 1	(reference)		(reference)	
Tertile 2	0.30 (0.05-0.55)	0.018	0.18 (-0.07-0.43)	0.16
Tertile 3	0.49 (0.23-0.75)	<0.001	0.32 (0.05-0.59)	0.021
Ln (α2M)	0.79 (0.28-1.31)	0.003	0.37 (-0.17-0.91)	0.18
C3				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.12 (-0.38-0.13)	0.35	-0.09 (-0.34-0.16)	0.49
Tertile 3	0.17 (-0.11-0.44)	0.23	0.27 (-0.01-0.54)	0.054
C3	0.05 (-0.49-0.58)	0.86	0.19 (-0.36-0.74)	0.51
Ferritin				
Tertile 1	(reference)		(reference)	
Tertile 2	0.02 (-0.22-0.27)	0.86	0.07 (-0.18-0.31)	0.60
Tertile 3	-0.13 (-0.39-0.13)	0.32	-0.05 (-0.32-0.21)	0.70
Ln (Ferritin)	-0.07 (-0.18-0.04)	0.22	-0.04 (-0.16-0.08)	0.51
Haptoglobin				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.05 (-0.30-0.20)	0.71	0.00 (-0.25-0.25)	1.00
Tertile 3	0.01 (-0.24-0.25)	0.96	0.05 (-0.21-0.30)	0.73
square root (haptoglobin)	0.09 (-0.17-0.35)	0.49	0.16 (-0.12-0.44)	0.27
PAI-1				
Tertile 1	(reference)		(reference)	
Tertile 2	-0.18 (-0.42-0.06)	0.15	-0.11 (-0.35-0.14)	0.39
Tertile 3	-0.11 (-0.36-0.13)	0.37	0.06 (-0.19-0.30)	0.66
Ln (PAI-1)	-0.07 (-0.23-0.09)	0.40	0.05 (-0.11-0.21)	0.56

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplementary table 3. Association of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with MACE

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
AAT						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.73 (0.81-3.66)	0.16	1.76 (0.83-3.74)	0.14	1.63 (0.76-3.49)	0.21
Tertile 3	1.36 (0.62-3.01)	0.44	1.37 (0.62-3.03)	0.44	1.00 (0.43-2.30)	0.99
LN (AAT)	1.71 (0.56-5.24)	0.33	1.78 (0.58-5.52)	0.32	0.76 (0.21-2.68)	0.67
α2M						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.36 (0.68-2.71)	0.39	1.22 (0.61-2.46)	0.58	1.46 (0.71-3.00)	0.30
Tertile 3	1.20 (0.57-2.52)	0.63	1.03 (0.48-2.23)	0.93	1.29 (0.59-2.79)	0.52
LN (α2M)	1.49 (0.39-5.63)	0.56	1.12 (0.27-4.71)	0.88	1.72 (0.44-6.65)	0.44
C3						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.26 (0.64-2.48)	0.51	1.33 (0.68-2.63)	0.41	1.23 (0.62-2.41)	0.56
Tertile 3	0.80 (0.38-1.68)	0.55	0.88 (0.41-1.87)	0.74	0.67 (0.31-1.43)	0.30
C3	0.86 (0.21-3.55)	0.84	1.12 (0.27-4.68)	0.88	0.44 (0.10-1.91)	0.27
Ferritin						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.32 (0.70-2.48)	0.39	1.32 (0.70-2.49)	0.40	1.33 (0.70-2.50)	0.38
Tertile 3	0.95 (0.48-1.88)	0.88	0.92 (0.46-1.85)	0.81	0.87 (0.43-1.77)	0.70
LN (Ferritin)	1.00 (0.75-1.35)	0.99	0.99 (0.73-1.34)	0.94	0.97 (0.72-1.32)	0.86
Haptoglobin						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.07 (0.55-2.10)	0.83	1.13 (0.58-2.22)	0.72	1.07 (0.54-2.09)	0.85
Tertile 3	1.36 (0.71-2.58)	0.35	1.48 (0.77-2.82)	0.24	1.10 (0.55-2.17)	0.79
square root (haptoglobin)	1.44 (0.74-2.78)	0.29	1.59 (0.81-3.12)	0.18	1.01 (0.48-2.09)	0.99
PAI-1						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.88 (0.48-1.63)	0.69	0.93 (0.50-1.72)	0.81	0.89 (0.48-1.64)	0.71
Tertile 3	0.67 (0.35-1.30)	0.24	0.77 (0.40-1.51)	0.45	0.67 (0.34-1.29)	0.23
LN (PAI-1)	0.82 (0.54-1.25)	0.36	0.93 (0.60-1.43)	0.74	0.82 (0.53-1.26)	0.37

*adjusted for age, gender and indication for coronary angiography

additionally adjusted for diabetes mellitus, hypertension and CRP

Chapter 8

Circulating chemokines in relation to coronary plaque characteristics on radiofrequency intravascular ultrasound and cardiovascular outcome

Cheng JM, Oemrawsingh RM, Akkerhuis KM, Garcia-Garcia HM, De Boer SP, Battes LC, Buljubasic N, Lenzen MJ, De Jaegere PP, Van Geuns RJ, Serruys PW, Kardys I, Boersma E.

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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

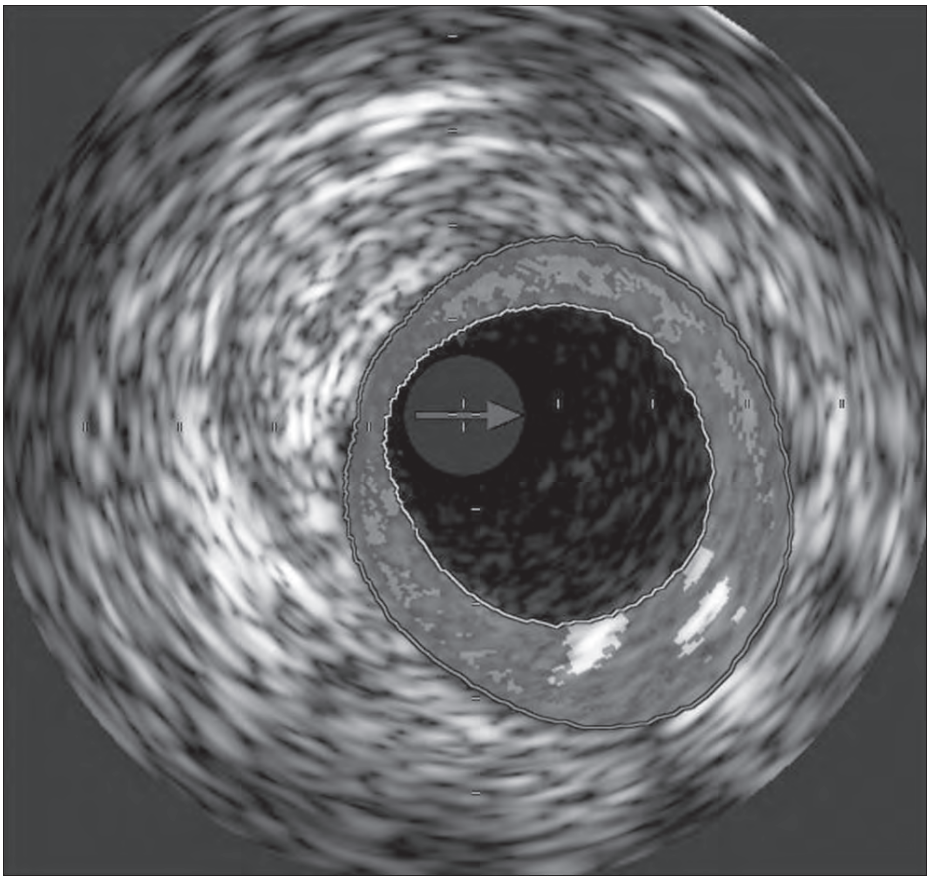

Figure 1. Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology
Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology is defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. White indicates dense calcium, light green indicates fibrofatty tissue, dark green indicates fibrous tissue.
See also Appendix: Color figure 3.

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. Baseline characteristics (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

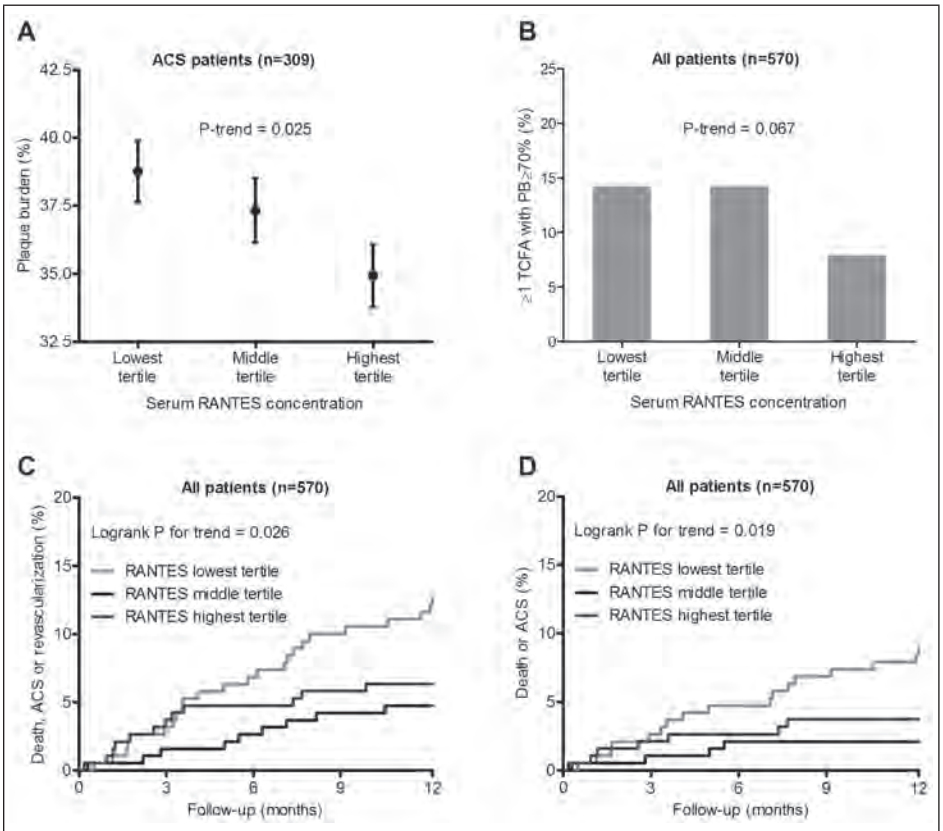

Figure 2. Associations of circulating RANTES concentrations with coronary atherosclerosis and clinical outcome

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.

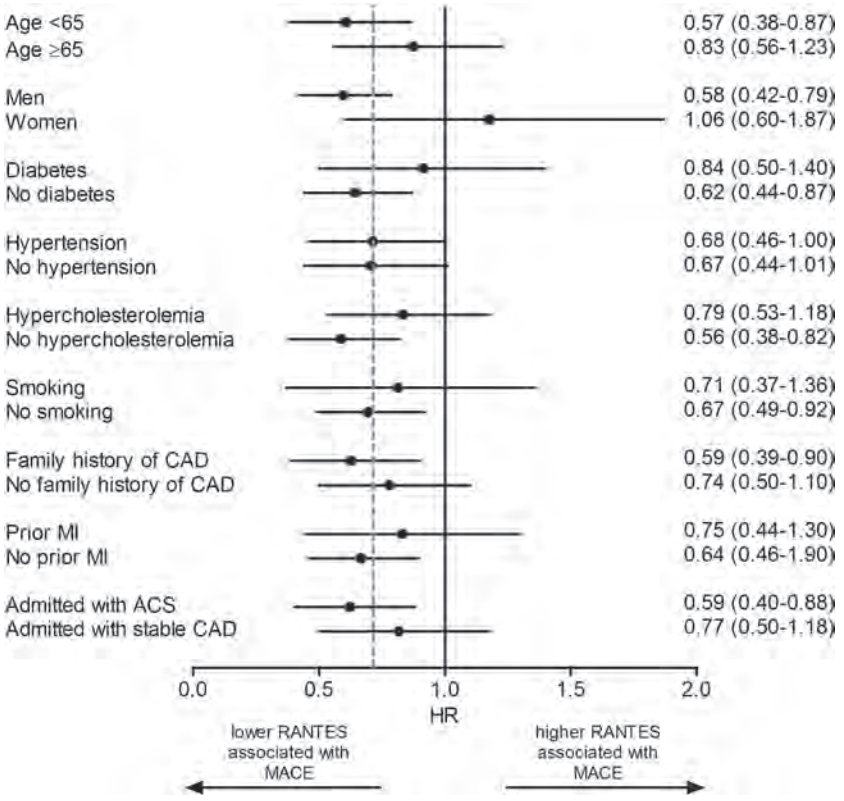

Figure 3. Association between RANTES level and major adverse cardiac events stratified by patient subgroups

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

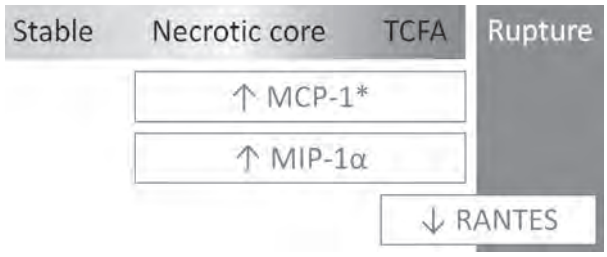

Figure 4. Circulating chemokine concentrations as biomarkers for phenotype of coronary atherosclerosis and risk of plaque rupture
Hypothesized model based on findings in this study. Plasma MCP-1, MIP-1α and RANTES concentrations were all associated with coronary plaque burden and different plaque phenotypes. 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

Chapter 9

Antibodies to periodontal pathogens are associated with coronary plaque remodeling but not with vulnerability or burden

De Boer SPM*, Cheng JM*, Rangé H, Garcia-Garcia HM, Heo JH, Akkerhuis KM, Meilhac O, Cosler G, Pussinen PJ, Van Geuns RJ, Serruys PW, Boersma E, Kardys I.

* These authors contributed equally.

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ABSTRACT

Objective: Previous studies have suggested positive associations between periodontal infection and cardiovascular disease. We aimed to investigate the associations of circulating antibodies against periodontal pathogens with 1-year cardiovascular outcome, as well as the extent of coronary atherosclerosis, plaque vulnerability and lesion remodeling on intravascular ultrasound (IVUS) imaging.

Methods: Between 2008 and 2011, radiofrequency IVUS imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography. Immunoglobulin G (IgG) and A (IgA) against *P.gingivalis*, *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia* were measured in plasma.

Results: None of the antibody levels were associated with coronary plaque burden, radiofreqeuncy-IVUS-derived thin-cap fibroatheroma lesion morphology or 1-year incidence of major adverse cardiac events (MACE), which included all-cause mortality, acute coronary syndrome and unplanned coronary revascularization. IgA against *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia* were inversely associated with extent of positive lesion remodeling (OR for highest versus lowest tertile 0.55, 95%CI 0.35-0.88, p=0.012; 0.53, 95%CI 0.32-0.87, p=0.012; and 0.64, 95%CI 0.40-1.02, p=0.061, respectively). In diabetic patients specifically, IgG against *P.gingivalis* tended to be associated with coronary plaque burden (p=0.080), while IgA against *P.gingivalis* tended to be associated with incident MACE (p=0.060).

Conclusion: Plasma IgG and IgA against major periodontal pathogens were not associated with the extent of coronary atherosclerosis (with the exception of a trend in diabetics) nor with coronary plaque vulnerability. IgA against periodontal pathogens were inversely associated with extent of coronary remodeling. Altogether, these results do not add evidence for a substantial role of systemic exposure to periodontal pathogens in coronary artery disease.

INTRODUCTION

Periodontitis is a bacterially induced chronic inflammatory disease of tissues supporting the teeth and is highly prevalent (20 to 50%) in the adult population.[1] In the past decades, several epidemiological studies have suggested positive associations between clinically established periodontal and cardiovascular disease,[2-4] and several small experimental studies have proposed potential mechanisms underlying these associations.[2,5] The mechanism that has been most advocated is bacteraemia followed by vascular contamination by periodontal pathogens.[2,6] On the other hand, there have also been many studies that failed to demonstrate such associations between periodontal infection and atherosclerosis, particularly after adjusting for confounding variables.[2] Furthermore, evidence that periodontal interventions or systemic antibiotic treatment result in improved cardiovascular outcomes is currently lacking.[2,7] Therefore, the proposed independent association between periodontal disease and atherosclerosis may still be considered as controversial.

This controversy is further underscored by the nature of the measures that have been used for periodontal infection (i.e. exposure) and atherosclerotic disease (i.e. outcome) in previous studies. The measures for periodontal infection were mostly subjective or based on clinical findings, usually in studies with limited sample size.[2] However, circulating immunoglobulin G (IgG) and immunoglobulin A (IgA) levels against periodontal pathogens may be more accurate measures of periodontal infection and its severity.[8,9] Furthermore, they may be used in large epidemiological studies. Major periodontal pathogens include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Prevotella intermedia*.[2] The outcome measures that have been used in previous studies mostly consisted of clinical diagnosis of coronary heart disease (such as history of myocardial infarction), which is a clinical manifestation of the underlying atherosclerosis, but may not be an accurately measure of the extent of atherosclerosis. Conversely, intravascular ultrasound (IVUS) imaging of the coronary arteries allows for accurate measurement of coronary plaque burden, as well as measurement of remodeling of coronary lesions.[10-12] Additionally, IVUS virtual histology (IVUS-VH) (i.e. analysis of IVUS radiofrequency backscatter), allows for tissue characterization and for identification of virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesions, which have previously been shown to be predictive for future coronary events.[10-15]

This study aims to investigate whether there are positive associations between plasma IgG and IgA-class immunoglobulin levels against four major periodontal pathogens (i.e. *P.gingivalis*, *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia*) and the extent of coronary atherosclerosis, coronary plaque vulnerability and coronary remodeling as measured by IVUS, as well as 1-year cardiovascular outcome.

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.[12,16] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) (n=318) or stable coronary artery disease (n=263) 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.

Antibodies against periodontal pathogens

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were further processed in the clinical laboratory of the Erasmus MC, and stored at a temperature of -80°C within 2 hours after blood collection. Subsequently, EDTA-plasma samples (n=575) were transported under controlled conditions (at a temperature of -80°C) to the Laboratory for Vascular Translational Science, INSERM UMRS 1148 , Paris, France, where levels of IgG and IgA against *A.actinomycetemcomitans*, *P.intermedia*, *P.gingivalis* and *T.forsythia* were measured. In 6 patients, EDTA samples were not available for measurement of antibodies against the periodontal pathogens.

Determination of antibodies against the periodontal bacteria were performed by enzyme-linked immuno sorbent assay (ELISA). One strain of P.intermedia, one strain of T.forsythia, a mixture of six A.actinomycetemcomitans strains and two P.gingivalis strains were used as antigens. The strains were: *P.intermedia* CIP 104480, *T.forsythia* CIP 105220, *A.actinomycetemcomitans* ATCC 29523, ATCC 43718, ATCC 33384, IDH 781, IDH 1705 and C59 (representing serotypes a, b, c, d, e and one nonserotypeable strain respectively) and *P.gingivalis* CIP 103683, OMGS 434 (representing serotypes a and c). The bacteria were cultivated in medium 20 (composition available on line at www.crbip.pasteur.fr) for the CIP strains and according to the multiserotype ELISA protocol published by Pussinen et al.[9] Briefly, the suspensions of fixed bacteria (0.5% formalin-PBS) were centrifuged (5500g for 15 minutes at 4°C) and washed 3 times in PBS. The pellets were resuspended in 0.05 M bicarbonate buffer to obtain an optical density of 0.15 at 580 nm. For serotype mixture, equal volumes of the six *A. actinomycetemcomitans* or two *P.gingivalis* strains were mixed and used to coat the 96-well plates. The bacteria were dispended in the plates and were incubated at 37°C for 4 hours and overnight at 4°C. The

excess bacteria were removed by washing in 0.05% Tween 20 in PBS and the unspecific binding was blocked by 5% bovine serum albumin in PBS at room temperature for 30 minutes. Two dilutions of each serum were used in each assay. The dilutions used were 1:1500 and 1: 3000 for *A.actinomycetemcomitans* and *P.intermedia*, 1:100 and 1:200 for *P.gingivalis*, 1:400 and 1:800 for *T.forsythia*. After washing, 100 µl of peroxidase-labelled goat anti-human IgG or IgA (dilution 1:30,000 in 1% BSA-PBS) were added to each well and incubated for 1 hour at room temperature. After washing, o-phenylenediamine dihydrochloride (SigmaFast™OPD) was used as substrate for peroxidase and the reaction was stopped with 0.5N H_2SO_4 before reading at 492 nm. On each plate, two dilutions of a high and a low control serum in duplicate were added to monitor for the inter-assay variations. The results are expressed in mean optical absorbances.

Coronary intravascular ultrasound imaging

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, data on anti-periodontal pathogens antibodies and clinical outcomes. The IVUS virtual histology analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software.

The extent, phenotype and remodeling of the coronary atherosclerosis were assessed. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Plaque burden was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area (Figure 1A). 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.[14] IVUS-VH derived thin-cap fibroatheroma (VH-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 (Figure 1B).[10-13,15] Remodeling of the plaque lesion was assessed by means of the remodeling 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

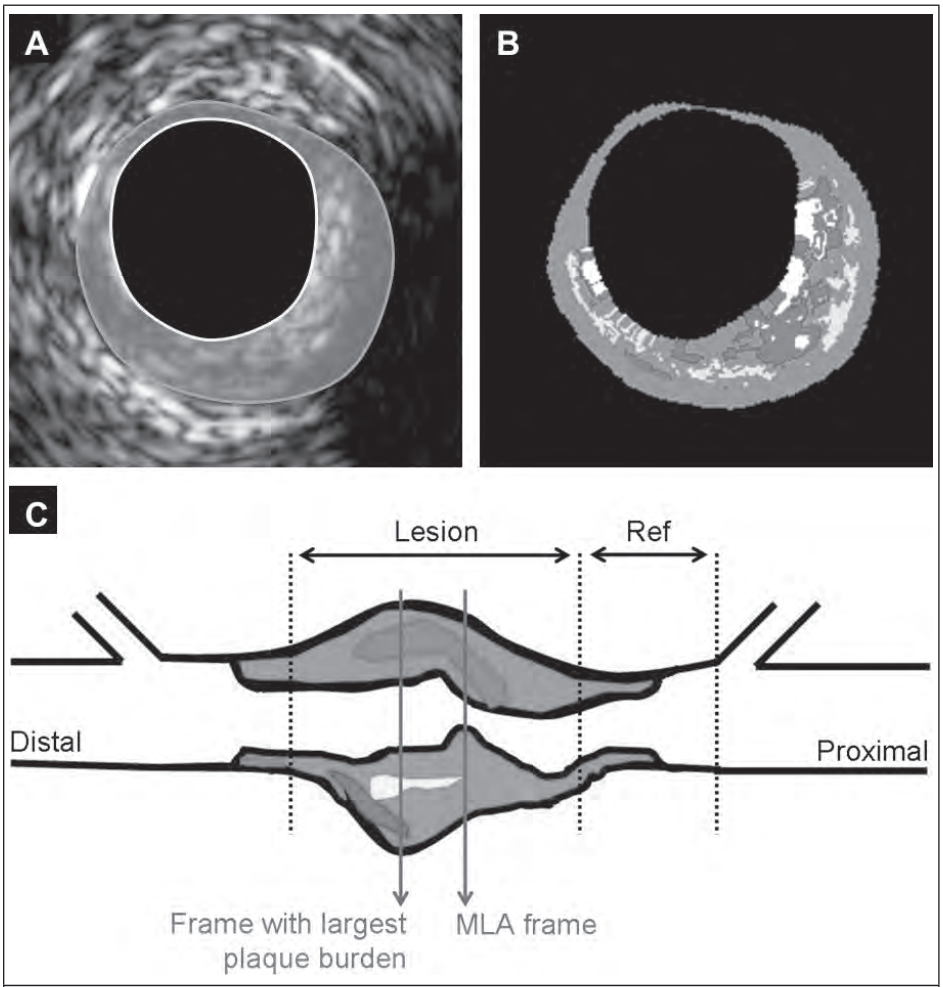

Figure 1. Methodology for intravascular ultrasound measurements
A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma lesion, defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. C: A lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Remodeling index was calculated by dividing the external elastic membrane cross-sectional area at the site of minimal luminal area (MLA) by the reference external elastic membrane cross-sectional area. Positive remodeling was defined as a remodeling index of >1.05.
See also Appendix: Color figure 4.

selected <10 mm proximal to the lesion. Positive remodeling (arterial expansion) was defined as a remodeling index of >1.05 (Figure 1C). Negative remodeling (arterial shrinkage) was defined as a remodeling index of <0.95.

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 contacted for additional information whenever necessary.

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.[17] Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). The endpoints were adjudicated by a clinical event committee that had no knowledge of the anti-periodontal pathogen antibodies and IVUS data.

Statistical analysis

The distributions of the continuous variables, including IgG and IgA levels against periodontal pathogens and the IVUS parameters, were tested for normality. Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are presented as median and interquartile range (IQR). IgG and IgA levels were not normally distributed and were therefore ln-transformed when analyzed as continuous variable. IgG and IgA levels were also analyzed when categorized into tertiles. Categorical variables are presented as numbers and percentages.

We examined associations of concentration of IgG and IgA against individual periodontal pathogens, as well as of the sum of all IgG and all IgA levels, with (1) plaque burden in the imaged coronary segment (a measure of the extent of coronary atherosclerosis), (2) VH-TCFA morphology of the coronary lesions (a measure of coronary plaque vulnerability), (3) positive lesion remodeling and (4) incident MACE during the follow-up period. Linear regression analyses were performed to evaluate the associations with plaque burden. The results are presented as β with 95% confidence interval (95% CI) per standard deviation increase in ln-transformed IgG or IgA level. Generalized estimating equation analyses were performed to evaluate the associations with VH-TCFA and positive lesion remodeling. This method accounts for the presence of multiple lesions within one individual patient. The results are presented as odds ratio (OR) with 95% CI. Cumulative event rates of MACE were estimated according to the Kaplan-Meier method. Cox proportional hazards regression analyses were performed to evaluate the associations with MACE. Patients lost to follow-up were considered at risk until the date

of last contact, at which time-point they were censored. The results are presented as hazard ratios (HR) with 95% confidence interval (95% CI).

In multivariable analyses, the variables age, gender, diabetes mellitus and smoking status (current smoking vs. never/past smoking) were considered as potential confounders and were entered into the full model. These variables were chosen because they were considered the most important confounders based on etiologic consideration and previous literature. Additionally, stratified analyses by smoking status and diabetes were performed to assess effect modification. 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 ± 11.3 years and 76% were men (Table 1). Coronary angiography was performed for various indications: 46% of patients had stable coronary artery disease, 26% of the patients had unstable angina pectoris and 28% of the patients had an acute myocardial infarction. ACS patients had slightly lower IgG-class antibody levels against *A. actinomycetemcomitans* compared to patients with stable coronary artery disease (Supplemental table 1). Antibody levels were similar in patients with and without multivessel disease (Supplemental table 1). The median length of the imaged coronary segment was 44.3 [33.9-55.4] mm. Mean plaque burden in the imaged coronary segment was 38.2 ± 11.5 percent. In the imaged coronary segments, a total of 723 coronary lesions have identified in 505 (87%) patients, including 271 VH-TCFA lesions in 240 (42%) patients.

Antibodies to periodontal pathogens and extent and vulnerability of coronary plaque

For reasons of conciseness, multivariable estimates are displayed in the tables. All univariable estimates are displayed in the supplement. In the total study population, plasma levels of IgG and IgA against *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *P. intermedia* were not associated with plaque burden in the imaged coronary segment (Table 2 and supplemental table 2). Plasma levels of IgG and IgA against *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *P. intermedia* were not associated with VH-TCFA lesion morphology either (Table 3 and supplemental table 3).

Table 1. **Baseline patient characteristics**		
		n = 575 patients
Clinical characteristics	Age, years	61.5 ± 11.3
	Men, n (%)	435 (75.7)
	Diabetes Mellitus, n (%)	99 (17.2)
	Hypertension. n (%)	299 (52.0)
	Hypercholesterolemia, n (%)	319 (55.5)
	Smoking, n (%)	165 (28.7)
	Positive family history, n (%)	298 (51.8)
	Previous MI, n (%)	183 (31.8)
	Previous PCI, n (%)	186 (32.3)
	Previous CABG, n (%)	18 (3.1)
	Previous stroke, n (%)	25 (4.3)
	History of peripheral artery disease, n (%)	36 (6.3)
	History of renal insufficiency, n (%)	32 (5.6)
	History of heart failure, n(%)	19 (3.3)
Plasma immunoglobulin levels	IgG against P. gingivalis, OD	0.61 [0.52-0.72]
	IgG against A. actinomycetemcomitans, OD	0.13 [0.08-0.19]
	IgG against T. forsythia, OD	1.00 [0.77-1.22]
	IgG against P. intermedia, OD	0.31 [0.18-0.55]
	IgA against P. gingivalis, OD	0.28 [0.17-0.47]
	IgA against A. actinomycetemcomitans, OD	0.16 [0.11-0.23]
	IgA against T. forsythia, OD	0.36 [0.19-0.60]
	IgA against P. intermedia, OD	0.10 [0.06-0.15]
Procedural characteristics	Indication for angiography	
	Acute MI, n (%)	163 (28.3)
	Unstable angina, n (%)	150 (26.1)
	Stable angina, n (%)	262 (45.6)
	Coronary artery disease*	
	No significant stenosis, n (%)	43 (7.5)
	1-vessel disease, n (%)	306 (53.2)
	2-vessel disease, n (%)	165 (28.7)
	3-vessel disease, n (%)	61 (10.6)
	PCI performed, n (%)	505 (87.8)
IVUS characteristics	Imaged coronary artery	
	Left anterior descending, n (%)	206 (35.8)
	Left circumflex, n (%)	193 (33.6)
	Right coronary artery, n (%)	176 (30.6)
	Median imaged segment length, mm	44.3 [33.9-55.4]
	Mean plaque burden, %	38.2 ± 11.5

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

Table 2. Association between periodontal pathogens and coronary plaque burden

		Adjusted β* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	-0.70 (-2.99 ; 1.60)	0.55
	tertile 3 vs. 1	1.21 (-1.07 ; -3.49)	0.30
	Linear association		0.62
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.04 (-1.27 ; 3.36)	0.38
	tertile 3 vs. 1	-1.06 (-3.28 ; 1.16)	0.35
	Linear association		0.70
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.01 (-2.22 ; 2.24)	0.99
	tertile 3 vs. 1	-0.04 (-2.35 ; 2.27)	0.97
	Linear association		0.89
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-2.33 (-4.61 ; -0.04)	0.046
	tertile 3 vs. 1	-0.22 (-2.49 ; 2.05)	0.85
	Linear association		0.69
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	-0.53 (-2.71 ; 1.66)	0.64
	tertile 3 vs. 1	-1.31 (-3.61 ; 0.99)	0.26
	Linear association		0.58
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.28 (-1.01 ; 3.58)	0.27
	tertile 3 vs. 1	-0.22 (-2.47 ; 2.02)	0.85
	Linear association		0.29
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	-1.24 (-3.53 ; 1.06)	0.29
	tertile 3 vs. 1	-1.68 (-3.87 ; 0.51)	0.13
	Linear association		0.27
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-1.90 (-4.17 ; 0.38)	0.10
	tertile 3 vs. 1	-0.79 (-2.98 ; 1.40)	0.48
	Linear association		0.56

* β indicates the increase in plaque burden per standard deviation increase in ln-transformed immunoglobulin level. Adjusted for age, gender, smoking and diabetes.

Table 3. Association between periodontal pathogens and thin-cap fibroatheroma morphology

		Adjusted OR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.99 (0.67-1.45)	0.94
	tertile 3 vs. 1	1.01 (0.68-1.49)	0.98
	Linear association		0.91
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.20 (0.82-1.76)	0.36
	tertile 3 vs. 1	0.85 (0.57-1.27)	0.43
	Linear association		0.52
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.01 (0.69-1.49)	0.95
	tertile 3 vs. 1	1.05 (0.71-1.56)	0.81
	Linear association		0.96
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.88 (0.60-1.30)	0.52
	tertile 3 vs. 1	0.95 (0.65-1.39)	0.80
	Linear association		0.45
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.81 (0.56-1.18)	0.28
	tertile 3 vs. 1	0.84 (0.57-1.25)	0.40
	Linear association		0.97
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.12 (0.77-1.64)	0.55
	tertile 3 vs. 1	0.78 (0.53-1.16)	0.22
	Linear association		0.32
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.91 (0.63-1.32)	0.62
	tertile 3 vs. 1	0.94 (0.64-1.40)	0.78
	Linear association		0.80
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.10 (0.75-1.61)	0.64
	tertile 3 vs. 1	1.16 (0.79-1.71)	0.44
	Linear association		0.92

* Adjusted for age, gender, smoking and diabetes.

Antibodies to periodontal pathogens and lesion remodeling

IgA-class antibody levels against *A. actinomycetemcomitans* were inversely associated with the extent of positive lesion remodeling (p for linear association 0.013) and directly with the extent of negative lesion remodeling (p for linear association 0.034). Similar associations with positive lesion remodeling were also seen with the highest tertiles of *T. forsythia* (p=0.012) and *P. intermedia* (p=0.012) IgA levels (Table 4, Figure 2, Supplemental table 4 and supplemental table 5).

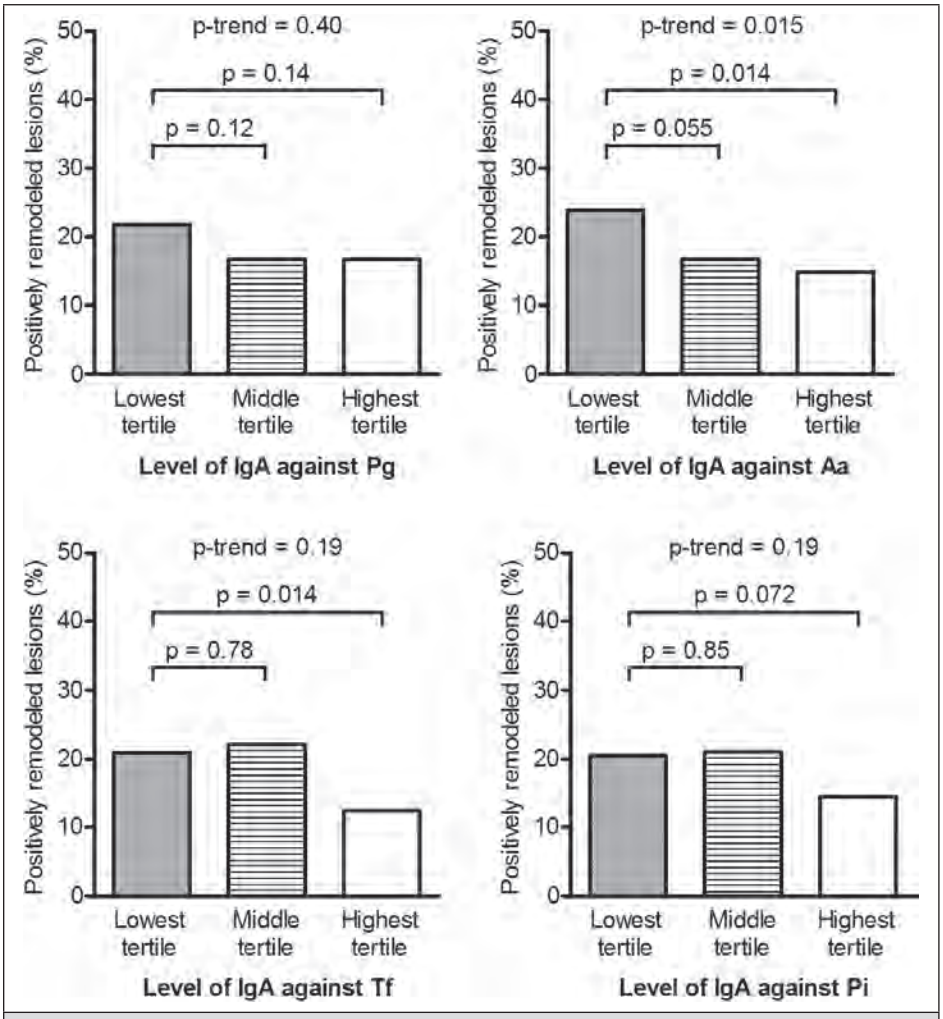

Figure 2. Association between level of immunoglobulin A against periodontal pathogens and positively remodeled lesions

Aa = Aggregatibacter actinomycetemcomitans; IgA = immunoglobulin A; Pi = Prevotella intermedia; Pg = Porphyromonas gingivalis; Tf = Tannerella forsythia.

Table 4. Association between periodontal pathogens and positive lesion remodeling

		Adjusted OR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	1.20 (0.75-1.92)	0.46
	tertile 3 vs. 1	1.07 (0.66-1.75)	0.79
	Linear association		0.36
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.71 (0.45-1.14)	0.71
	tertile 3 vs. 1	0.74 (0.47-1.19)	0.22
	Linear association		0.18
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.17 (0.74-1.83)	0.50
	tertile 3 vs. 1	0.75 (0.46-1.25)	0.27
	Linear association		0.63
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.81 (0.51-1.28)	0.37
	tertile 3 vs. 1	0.73 (0.46-1.15)	0.18
	Linear association		0.14
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.72 (0.45-1.13)	0.15
	tertile 3 vs. 1	0.71 (0.44-1.14)	0.15
	Linear association		0.42
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.63 (0.39-1.00)	0.048
	tertile 3 vs. 1	0.55 (0.35-0.88)	0.012
	Linear association		0.013
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.08 (0.69-1.67)	0.74
	tertile 3 vs. 1	0.53 (0.32-0.87)	0.012
	Linear association		0.19
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.05 (0.66-1.66)	0.85
	tertile 3 vs. 1	0.64 (0.40-1.02)	0.061
	Linear association		0.20

* Adjusted for age, gender, smoking and diabetes.

Antibodies to periodontal pathogens and cardiovascular outcome

Vital status at 1-year follow-up could be acquired for 573 (99.7%) patients. Response rate of the questionnaires that were sent to all living patients was 92.7%. After 1 year of follow-up, 56 patients had experienced a MACE. The cumulative Kaplan-Meier incidences

of the 30-day, 6-month and 1-year MACE were 0.9%, 5.6%, and 9.8%, respectively. High plasma concentrations of IgG and IgA against *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *P. intermedia* were not associated with the occurrence of MACE during 1 year of follow-up (Table 5 and supplemental table 6).

Table 5. Association between periodontal pathogens and 1-year major adverse cardiac events

		Adjusted HR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.85 (0.43-1.68)	0.63
	tertile 3 vs. 1	1.03 (0.55-1.92)	0.94
	Linear association		0.95
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.31 (0.69-2.49)	0.41
	tertile 3 vs. 1	0.91 (0.46-1.79)	0.91
	Linear association		1.00
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.51 (0.26-1.00)	0.048
	tertile 3 vs. 1	0.68 (0.36-1.27)	0.23
	Linear association		0.96
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.67 (0.90-3.08)	0.10
	tertile 3 vs. 1	0.63 (0.29-1.36)	0.24
	Linear association		0.91
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.92 (0.48-1.77)	0.81
	tertile 3 vs. 1	0.87 (0.46-1.67)	0.68
	Linear association		0.67
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.78 (0.92-3.46)	0.087
	tertile 3 vs. 1	1.19 (0.58-2.41)	0.64
	Linear association		0.65
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.81 (0.41-1.60)	0.55
	tertile 3 vs. 1	1.13 (0.59-2.14)	0.72
	Linear association		0.91
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.20 (0.62-2.34)	0.59
	tertile 3 vs. 1	1.18 (0.61-2.28)	0.63
	Linear association		0.97

* Adjusted for age, gender, smoking and diabetes.

Sum of IgG and IgA levels

The sum of IgG levels and the sum of IgA levels were neither associated with coronary plaque burden (p=0.86 and p=0.30 respectively), nor with VH-TCFA lesion morphology (p=0.60 and p=0.97 respectively), nor with MACE (p=0.15 and p=0.67 respectively). The sum of IgA levels was inversely associated with the extent of positive lesion remodeling (highest versus lowest tertile OR 0.50, 95% CI 0.31-0.82, p=0.006), while the sum of IgG levels was not (p=0.68).

Analyses stratified by diabetes and smoking status

In patients with diabetes, the highest IgG tertile against *P. gingivalis* was associated with coronary plaque burden (p=0.020) while the corresponding IgA antibody levels tended to associate with MACE (p=0.16) (Figure 3). Although statistical significance mostly disappeared because of lower power in the subgroups, the estimates of the remaining associations that were found in the total study population remained materially the same after stratification on diabetes. The same applied to the estimates found in smokers and non-smokers.

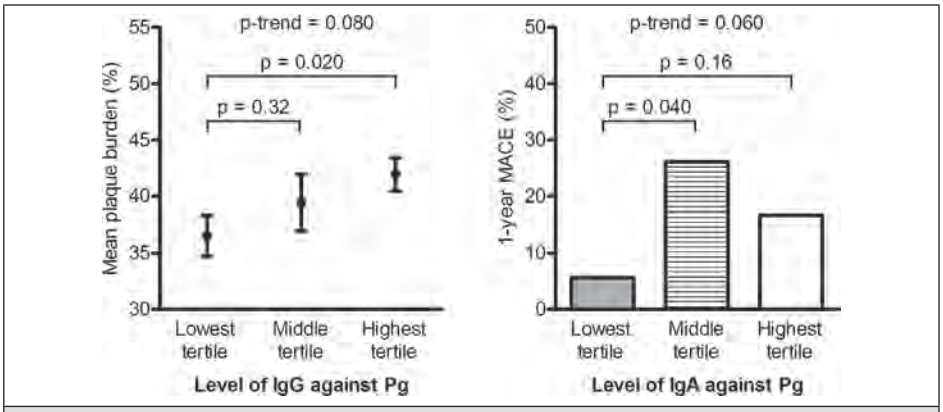

Figure 3. Associations of Porphyromonas gingivalis with extent of coronary atherosclerosis and with cardiovascular outcome in patients with diabetes
IgA = immunoglobulin A; IgG = immunoglobulin G; MACE = major adverse cardiac event; Pg = Porphyromonas gingivalis

DISCUSSION

This study investigated the associations between antibodies to major periodontal pathogens and coronary atherosclerosis on IVUS-VH. We found that IgG and IgA against *P.gingivalis*, *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia* were not associated with coronary plaque burden or VH-TCFA lesion morphology in the overall study popula-

tion. High levels of IgA against *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia*, however, were associated with lower extent of positive lesion remodeling. The serologic markers of periodontal infection were not predictive for 1-year MACE. In stratified analyses, antibodies against *P.gingivalis* tended to be associated with the extent of coronary atherosclerosis and with incident MACE during 1 year follow-up in patients with diabetes.

The major strengths of this study include the objective measurements of the exposure (i.e. periodontal infection as assessed by antibodies), the precise measurement of the extent of coronary atherosclerosis, as well as the assessment of the composition of coronary atherosclerosis (atherosclerotic plaque morphology and lesion remodeling). The validated multiserotype ELISAs that have been used for the measurement of *P.gingivalis* and *A. actinomycetemcomitans* antibodies have a good sensitivity (71%) and specificity (90%) for clinically and radiographically diagnosed periodontitis, and allowed us to measure the infection by these periodontal pathogens.[18] IVUS enables for accurate measurements of various coronary plaque characteristics, including plaque burden, plaque morphology and lesion remodeling.[12,16] To our best knowledge, this is the first study on periodontal disease that used IVUS to measure the extent of coronary atherosclerosis, and the associations between periodontal infection and plaque morphology and lesion remodeling have so far not been investigated at all.

Many observational studies have investigated the association between periodontal disease and cardiovascular disease; the latter mostly defined as extent of atherosclerosis or occurrence of clinical cardiovascular events.[2] However, in these studies, periodontal disease has been broadly defined by a variety of measures, including self-reported assessment of tooth loss or periodontal status, and clinically or radiographically assessed gingival inflammation or pathological periodontal pockets. Few studies have assessed the periodontal infection by measuring IgG and IgA antibody levels against several bacteria.[19-26] A study by Colhoun et al. found an association between IgG against *P.gingivalis* and *A.actinomycetemcomitans* and coronary artery calcification as measured by computed tomography in diabetic subjects, although this association did not appear to be independent of baseline characteristics.[20] In line with this finding, our study showed an association between IgG against *P.gingivalis* and the extent of coronary atherosclerosis on IVUS in diabetic patients with established coronary artery disease. Cross-sectional and case-control studies by Pussinen et al., Beck et al, and Spahr et al. have reported positive associations between antibody levels against various periodontal pathogens and the presence of coronary heart disease within large population-based cohorts.[19,23-26] Furthermore, two prospective studies have investigated the association between serum antibody titers and risk of incident MACE during follow-up. Lund Haheim et al. measured IgG to *P.gingivalis*, *A.actinomycetemcomitans*, *T.forsythia*, and *Treponema denticola*, and found that no single bacterium but rather combinations were related to increasing risk for incident myocardial infarction.[21] Pussinen et al, measured IgG and IgA to *P.gingivalis*

and *A.actinomycetemcomitans*, and found that IgA against *P. gingivalis* was associated with increased risk for occurrence of myocardial infarction during 10 years of follow-up.[24] The latter is in line with our finding that IgA against *P.gingivalis* is associated with 1-year MACE in diabetic patients with coronary artery disease. The lack of further associations with extent of coronary atherosclerosis and with MACE in our full cohort may be explained by the fact that ours was a cohort of patients with known coronary artery disease, and associations may be different compared to population-based cohorts or case-control studies, which contain healthy control subjects.

In the current study, we did not find an association between antibody levels against periodontal pathogens and VH-TCFA lesion morphology, which suggests that periodontal infection and its inflammatory reaction is not associated with coronary plaque vulnerability. However, we did find that IgA against *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia* (but not *P.gingivalis*) were inversely associated with extent of positive lesion remodeling. Modest positive lesion remodeling may be considered as a physiological, and thus favourable, response to progression of atherosclerotic plaque, also known as the Glagov adaptive phenomenon.[27] In this light, infection with *A.actinomycetemcomitans*, *T.forsythia* and *P.intermedia* may be considered as being associated with a lower adaptive capacity to atherosclerotic burden.

Some limitations of this study need to be acknowledged. Firstly, clinical periodontal examination was not performed in our study. Although measurement of antibodies provides good sensitivity and specificity for clinically and radiographically diagnosed periodontitis, data on actual presence of periodontitis or history of periodontal destruction is lacking. Furthermore, IgG and IgA levels reflect both past and recent exposure to the periodontal pathogens.[28] In the current study, we could not investigate the effects of the active phase of periodontitis, which can only be determined by clinical measurements of changes in attachment level, on coronary atherosclerosis.[8] The ideal analysis includes both the clinical diagnosis of periodontitis and the antibodies against periodontal pathogens.

Secondly, a single non-culprit coronary vessel was imaged in this study. This approach was chosen based on the hypothesis that the phenotype of a non-culprit artery segment represents the patient's systemic atherosclerotic disease burden.[16] Although this hypothesis may be debated, it is highly supported by our previous finding that IVUS in only one non-culprit vessel has strong prognostic value.[12] Finally, the spatial resolution of IVUS-VH (150μm) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65μm).[29] Therefore, IVUS-VH tends to over-estimate the number of histopathological TCFA lesions. Nevertheless, the presence of VH-TCFA lesions has been shown to have prognostic information.[10-12]

In conclusion, in the current study, plasma levels of IgG and IgA against four major periodontal pathogens were neither associated with the extent of coronary atherosclerosis,

nor with coronary plaque vulnerability, as measured with IVUS-VH in a population of patients with established coronary artery disease. IgA against *A.actinomycetemcomitans*, *T.forsythia* and *P.Intermedia* were inversely associated with extent of positive coronary remodeling. IgG and IgA against the four major periodontal pathogens that we tested were not predictive for cardiovascular outcome at 1 year. Only in the subgroup of patients with diabetes, antibodies against *P.gingivalis* tended to be associated with the extent of coronary atherosclerosis and with incident MACE during 1-year follow-up. Altogether, the results of the current study do not add evidence for the hypothesis that systemic exposure to periodontal pathogens, as assessed by antibodies against periodontal pathogens, plays a substantial role in coronary artery disease. Further studies in diabetic patients are needed to clarify the potential role of periodontal pathogens in coronary atherogenesis and associated cardiovascular events.

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SUPPLEMENTAL TABLES

Supplemental table 1. Antibody levels in acute coronary syndrome versus stable coronary artery disease and in patients with and without multivessel disease

	ACS	Stable CAD	P
IgG against P. gingivalis, OD	0.61 [0.51-0.71]	0.61 [0.53-0.72]	0.39
IgG against A. actinomycetemcomitans, OD	0.12 [0.08-0.18]	0.14 [0.09-0.21]	0.031
IgG against T. forsythia, OD	1.01 [0.82-1.23]	0.98 [0.76-1.22]	0.38
IgG against P. intermedia, OD	0.30 [0.18-0.55]	0.31 [0.17-0.54]	0.87
IgA against P. gingivalis, OD	0.29 [0.17-0.48]	0.28 [0.17-0.45]	0.41
IgA against A. actinomycetemcomitans, OD	0.16 [0.11-0.24]	0.16 [0.12-0.23]	0.92
IgA against T. forsythia, OD	0.39 [0.19-0.59]	0.34 [0.19-0.61]	0.92
IgA against P. intermedia, OD	0.10 [0.06-0.16]	0.09 [0.06-0.15]	0.52
	Multivessel disease	**No multivessel disease**	**P**
IgG against P. gingivalis, OD	0.61 [0.53-0.71]	0.61 [0.50-0.76]	0.69
IgG against A. actinomycetemcomitans, OD	0.13 [0.08-0.20]	0.13 [0.09-0.18]	0.93
IgG against T. forsythia, OD	1.00 [0.82-1.22]	0.98 [0.73-1.23]	0.32
IgG against P. intermedia, OD	0.32 [0.19-0.56]	0.28 [0.17-0.53]	0.34
IgA against P. gingivalis, OD	0.28 [0.16-0.46]	0.28 [0.17-0.49]	0.49
IgA against A. actinomycetemcomitans, OD	0.16 [0.11-0.24]	0.16 [0.12-0.23]	0.31
IgA against T. forsythia, OD	0.36 [0.19-0.59]	0.37 [0.19-0.61]	0.96
IgA against P. intermedia, OD	0.10 [0.06-0.16]	0.08 [0.05-0.15]	0.052

ACS = acute coronary syndrome; CAD = stable coronary artery disease.

Supplemental table 2. Association between periodontal pathogens and coronary plaque burden in univariable analysis

		Unadjusted β* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	-0.67 (-3.01 ; 1.67)	0.57
	tertile 3 vs. 1	1.66 (-0.63 ; 3.94)	0.16
	Linear association		0.36
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.79 (-1.60 ; 3.18)	0.52
	tertile 3 vs. 1	-0.42 (-2.70 ; 1.87)	0.72
	Linear association		0.46
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	-0.42 (2.73 ; 1.89)	0.72
	tertile 3 vs. 1	-0.26 (-2.55 ; 2.03)	0.82
	Linear association		0.63
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-2.08 (-4.41 ; 0.25)	0.08
	tertile 3 vs. 1	-0.51 (-2.79 ; 1.77)	0.66
	Linear association		0.97
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.58 (-1.65 ; 2.81)	0.61
	tertile 3 vs. 1	-0.74 (-3.08 ; 1.60)	0.53
	Linear association		0.91
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.79 (-0.56 ; 4.14)	0.14
	tertile 3 vs. 1	0.05 (-2.25 ; 2.36)	0.96
	Linear association		0.16
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	-0.45 (-2.80 ; 1.89)	0.71
	tertile 3 vs. 1	-0.85 (-3.10 ; 1.39)	0.46
	Linear association		0.37
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-1.80 (-4.12 ; 0.51)	0.13
	tertile 3 vs. 1	-0.28 (-2.55 ; 1.98)	0.81
	Linear association		0.95

* β indicates the increase in plaque burden per standard deviation increase in ln-transformed immunoglobulin level.
IgA = immunoglobulin A; IgG = immunoglobulin G.

Supplemental table 3. Association between periodontal pathogens and thin-cap fibroatheroma morphology in univariable analysis

		Unadjusted OR (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	1.01 (0.69-1.48)	0.95
	tertile 3 vs. 1	1.01 (0.69-1.49)	0.96
	Linear association		0.99
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.22 (0.83-1.79)	0.30
	tertile 3 vs. 1	0.88 (0.59-1.30)	0.51
	Linear association		0.63
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.05 (0.72-1.54)	0.81
	tertile 3 vs. 1	1.13 (0.77-1.67)	0.53
	Linear association		0.60
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.87 (0.59-1.28)	0.48
	tertile 3 vs. 1	1.01 (0.69-1.47)	0.98
	Linear association		0.70
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.79 (0.54-1.15)	0.22
	tertile 3 vs. 1	0.84 (0.57-1.25)	0.40
	Linear association		0.95
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.11 (0.76-1.62)	0.59
	tertile 3 vs. 1	0.78 (0.53-1.14)	0.20
	Linear association		0.30
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.89 (0.61-1.29)	0.53
	tertile 3 vs. 1	0.93 (0.63-1.38)	0.73
	Linear association		0.78
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.11 (0.76-1.64)	0.59
	tertile 3 vs. 1	1.17 (0.80-1.71)	0.43
	Linear association		0.90

IgA = immunoglobulin A; IgG = immunoglobulin G.

Supplemental table 4. Association between periodontal pathogens and positive lesion remodeling in univariable analysis

		Unadjusted OR (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	1.21 (0.76-1.94)	0.43
	tertile 3 vs. 1	1.08 (0.67-1.76)	0.75
	Linear association		0.34
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.73 (0.45-1.16)	0.18
	tertile 3 vs. 1	0.77 (0.48-1.22	0.26
	Linear association		0.22
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.21 (0.77-1.89)	0.41
	tertile 3 vs. 1	0.82 (0.50-1.33)	0.41
	Linear association		0.42
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.80 (0.51-1.28)	0.36
	tertile 3 vs. 1	0.76 (0.49-1.20)	0.24
	Linear association		0.21
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.70 (0.44-1.10)	0.12
	tertile 3 vs. 1	0.70 (0.44-1.12)	0.14
	Linear association		0.40
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.64 (0.41-1.01)	0.055
	tertile 3 vs. 1	0.56 (0.35-0.89)	0.014
	Linear association		0.015
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.07 (0.69-1.65)	0.78
	tertile 3 vs. 1	0.54 (0.33-0.88)	0.014
	Linear association		0.19
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.04 (0.66-1.64)	0.85
	tertile 3 vs. 1	0.66 (0.41-1.04)	0.072
	Linear association		0.19

IgA = immunoglobulin A; IgG = immunoglobulin G.

Supplemental table 5. Association between periodontal pathogens and negative lesion remodeling

		Unadjusted OR (95% CI)	P	Adjusted OR* (95% CI)	P
IgG against	tertile 1	reference		reference	
P.gingivalis	tertile 2vs.1	1.28 (0.89-1.85)	0.18	1.31 (0.91-1.88)	0.15
	tertile 3vs.1	0.96 (0.66-1.40)	0.85	0.97 (0.66-1.42)	0.86
	Linear association		0.54		0.59
IgG against	tertile 1	reference		reference	
A.actinomycetemcomitans	tertile 2vs.1	1.33 (0.93-1.92)	0.12	1.32 (0.92-1.90)	0.13
	tertile 3vs.1	1.31 (0.90-1.90)	0.15	1.33 (0.92-1.92)	0.14
	Linear association		0.14		0.11
IgG against	tertile 1	reference		reference	
T.forsythia	tertile 2vs.1	1.15 (0.80-1.65)	0.46	1.14 (0.79-1.64)	0.48
	tertile 3vs.1	1.07 (0.74-1.56)	0.72	1.07 (0.73-1.57)	0.73
	Linear association		0.83		0.85
IgG against	tertile 1	reference		reference	
P.intermedia	tertile 2vs.1	1.24 (0.86-1.78)	0.25	1.27 (0.88-1.82)	0.20
	tertile 3vs.1	1.07 (0.74-1.57)	0.71	1.08 (0.74-1.58)	0.70
	Linear association		0.53		0.47
IgA against	tertile 1	reference		reference	
P.gingivalis	tertile 2vs.1	1.18 (0.81-1.72)	0.39	1.22 (0.83-1.79)	0.31
	tertile 3vs.1	0.92 (0.63-1.35)	0.67	0.93 (0.64-1.36)	0.93
	Linear association		0.44		0.47
IgA against	tertile 1	reference		reference	
A.actinomycetemcomitans	tertile 2vs.1	1.03 (0.71-1.49)	0.89	1.05 (0.72-1.53)	0.82
	tertile 3vs.1	1.30 (0.90-1.88)	0.16	1.32 (0.91-1.90)	0.14
	Linear association		0.048		0.034
IgA against	tertile 1	reference		reference	
T.forsythia	tertile 2vs.1	1.07 (0.74-1.54)	0.73	1.08 (0.75-1.57)	0.67
	tertile 3vs.1	1.20 (0.83-1.75)	0.33	1.23 (0.85-1.79)	0.28
	Linear association		0.78		0.73
IgA against	tertile 1	reference		reference	
P.intermedia	tertile 2vs.1	0.91 (0.63-1.31)	0.60	0.92 (0.63-1.33)	0.65
	tertile 3vs.1	1.04 (0.72-1.50)	0.83	1.06 (0.74-1.53)	0.74
	Linear association		0.59		0.50

* Adjusted for age, gender, smoking and diabetes.
IgA = immunoglobulin A; IgG = immunoglobulin G.

Supplemental table 6. Association between periodontal pathogens and 1-year major adverse cardiac events in univariable analysis

		Unadjusted HR (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.87 (0.45-1.70)	0.69
	tertile 3 vs. 1	1.08 (0.58-2.02)	0.80
	Linear association		0.90
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.41 (0.74-2.66)	0.30
	tertile 3 vs. 1	1.00 (0.51-1.98)	0.99
	Linear association		0.76
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.45 (0.23-0.90)	0.025
	tertile 3 vs. 1	0.72 (0.40-1.30)	0.28
	Linear association		0.76
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.62 (0.88-2.96)	0.12
	tertile 3 vs. 1	0.69 (0.33-1.44)	0.33
	Linear association		0.38
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.90 (0.47-1.73)	0.76
	tertile 3 vs. 1	0.96 (0.51-1.80)	0.90
	Linear association		0.49
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.68 (0.88-3.21)	0.11
	tertile 3 vs. 1	1.14 (0.57-2.28)	0.71
	Linear association		0.71
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.80 (0.41-1.56)	0.51
	tertile 3 vs. 1	1.03 (0.55-1.91)	0.93
	Linear association		0.90
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.23 (0.64-2.36)	0.54
	tertile 3 vs. 1	1.22 (0.64-2.33)	0.55
	Linear association		0.78

IgA = immunoglobulin A; IgG = immunoglobulin G.

Chapter 10

PCSK9 in relation to coronary plaque inflammation and cardiovascular outcome

Cheng JM*, Oemrawsingh RM*, Garcia-Garcia HM, Boersma E, Van Geuns RJ, Serruys PW, Kardys I, Akkerhuis KM.

* These authors contributed equally.

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ABSTRACT

Background: 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.

Objectives: This study aims to investigate the association between serum PCSK9 and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque, 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, serum PCSK9 level was 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 (p=0.033), but was 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). High PCSK9 levels were associated with the composite endpoint of death or ACS (definite culprit lesion-related events not included as endpoints) during 1 year of follow-up (HR 1.42 per 100μg/L increase, 95%CI 1.02-1.99, p=0.040).

Conclusions: The serum PCSK9 level is linearly associated with the fraction and amount of necrotic core tissue in coronary atherosclerosis and with 1-year clinical outcome after coronary angiography, independently of serum LDL level and statin use. Therefore, PCSK9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL regulation.

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 study the effects of PCSK9 inhibition on LDL 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 the serum PCSK9 level and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS-VH imaging, as well as to assess the relation between serum PCSK9 level and 1-year cardiovascular outcome.

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.(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 between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. None of the patients were treated with drugs targeting PCSK9 during the study period. 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.

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., Minneapolis, 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. 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-VH data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS-VH catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second. The IVUS-VH images were sent to an independent core laboratory (Cardialysis bv, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for patient characteristics, PCSK9 levels and clinical outcome data. The 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 (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)

Follow-up and 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

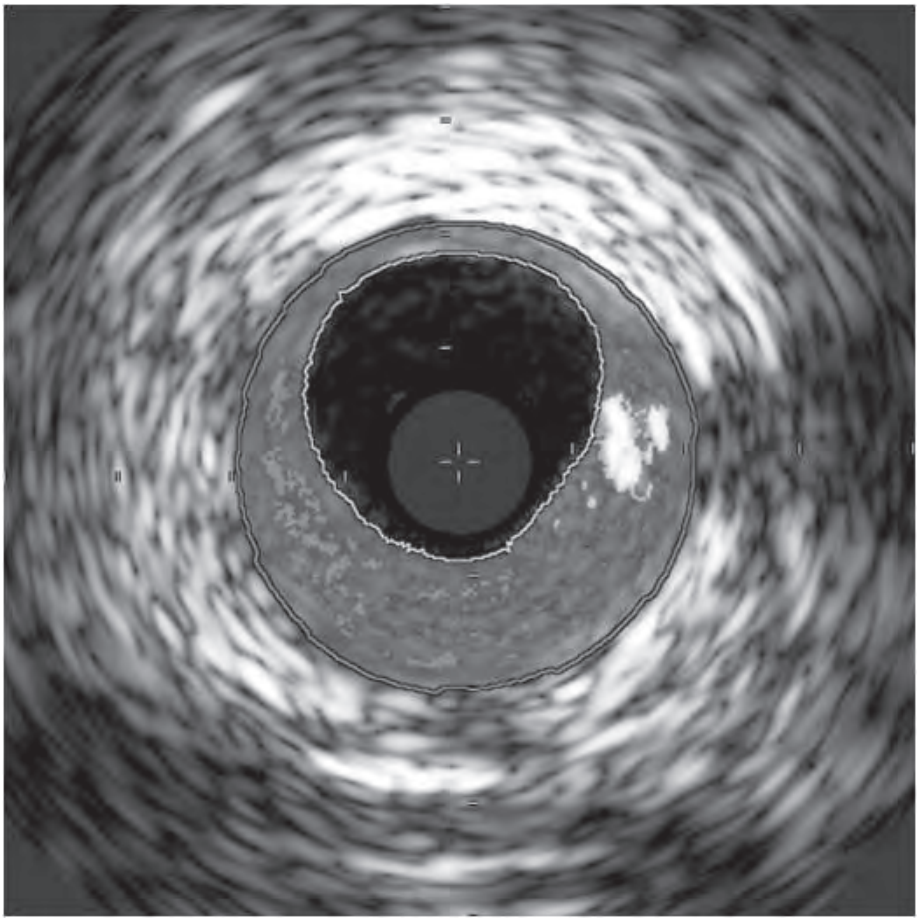

Figure 1. Intravascular ultrasound virtual histology imaging
Intravascular ultrasound virtual histology imaging was used to characterize atherosclerotic plaques into 4 different tissue types: fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red).
See also Appendix: Color figure 5.

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 the 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, the event was classified as indeterminate.

Occurrence of culprit lesion-related events is most likely caused by in-stent restenosis or in-stent thrombosis. Since, in the current study, we were specifically interested in unanticipated, spontaneous MACE, we took into account only those clinical endpoints that were defined as non-culprit lesion-related or indeterminate. These clinical endpoints were: 1. MACE, defined as non-culprit lesion-related or indeterminate mortality, ACS or unplanned coronary revascularization; and 2. the composite of non-culprit lesion-related or indeterminate mortality or ACS, both during 1 year of follow-up. Thus, definite culprit lesion-related events were not included in the clinical endpoints in this study. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction, non-ST segment elevation myocardial infarction 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). The endpoints were adjudicated by a clinical event committee that had no knowledge of the PCSK9 and IVUS-VH data.

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. Plaque volume and necrotic core volume were not normally distributed, and were therefore square root-transformed where after normality was reached. Linear regression analyses were performed to evaluate the associations of serum PCSK9 level 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 level 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 multivariable 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 level was additionally entered into the model to evaluate whether the associations between PCSK9 and coronary plaque characteristics were independent of serum LDL level. Additionally, stratified analyses were performed to evaluate 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).

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 PCSK9 and clinical study endpoints. The results are presented as unadjusted and adjusted hazard ratios (HR) with 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

Baseline characteristics

Mean age of the patients was 61.5 ± 11.3 years, 76% were men, and 55% had ACS (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

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)

Table 1. Baseline characteristics (continued)	
	n = 576 patients
History of heart failure, n (%)	19 (3.3)
Serum LDL cholesterol, mmol/L	2.72 [2.12-3.54]
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.

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 and coronary plaque characteristics

In univariable 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 in PCSK9, 95% CI 0.63-1.99, p<0.001) and tended to be associated with a higher absolute necrotic core volume (p=0.075) (Table 2 and Figure 2). Furthermore, PCSK9 levels were inversely associated with fractions of fibrous tissue (p=0.004) and fibro-fatty tissue (p=0.002), and positively associated with dense calcium fraction (p=0.004) (Supplemental Figure 1). PCSK9 levels were not associated with overall plaque burden (p=0.22), plaque volume (p=0.48) or the presence of IVUS-VH-derived TCFA lesions (p=0.93). After adjustment in multivariable analysis, PCSK9 levels were both significantly associated with necrotic core fraction (β = 1.22 percent increase in necrotic core per 100 μg/L increase in

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.

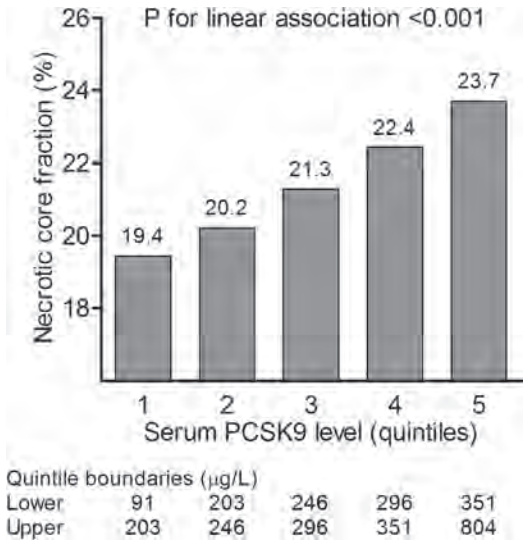

Figure 2. Association between serum PCSK9 level and fraction of coronary plaque that consists of necrotic core tissue
PCSK9 = proprotein convertase substilisin/kexin type 9.

PCSK9, 95% CI 0.52-1.91, p<0.001) and absolute necrotic core volume (p=0.036). These associations did not change materially after additional adjustment for serum LDL level. Subgroup analysis showed that the positive association between serum PCSK9 level 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. (Figure 3). There was no significant heterogeneity in the β estimate between the evaluated patient subgroups.

Incident major adverse cardiac events

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 3). 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 MACE (definite culprit lesion-related events not included as endpoint) were 0.7%, 4.7%, and 7.8%, respectively. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year composite of death or ACS (definite culprit lesion-related events not included) were 0.7%, 3.1%, and 4.8%, respectively.

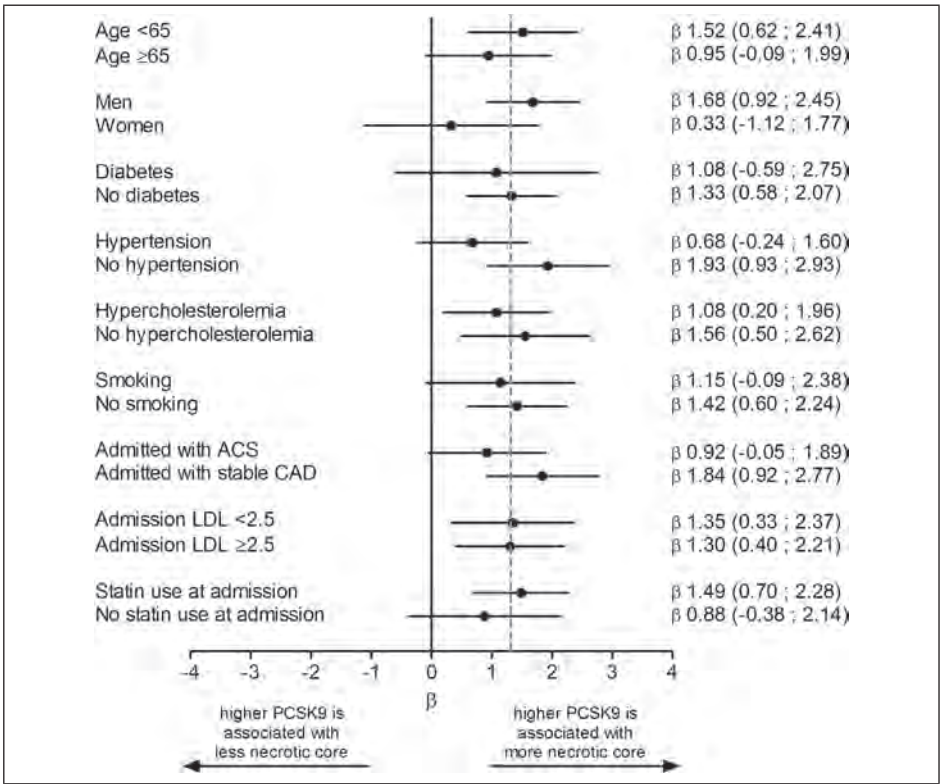

Figure 3. Association between PCSK9 level and necrotic core fraction stratified by patient subgroups

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.

Table 3. Incidence of 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 major adverse cardiac events, 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

*Study endpoints

Association between PCSK9 and cardiovascular outcome

In univariable analysis, serum PCSK9 level was only significantly associated with the composite of death or ACS when PCSK9 was analyzed as a categorical variable (PCSK9 above median 3.1% versus below median 6.6%, p=0.049) (Figure 4). Serum PCSK9 levels were not associated with MACE (p=0.50) or the composite of death or ACS (p=0.17) when PCSK9 was analyzed as a continuous variable (Table 4). After adjustment in multivariable analysis, however, higher serum PCSK9 levels were significantly associated with a higher rate of death and ACS (HR 1.43 per 100 µg/L increase in serum PCSK9 level, 95%

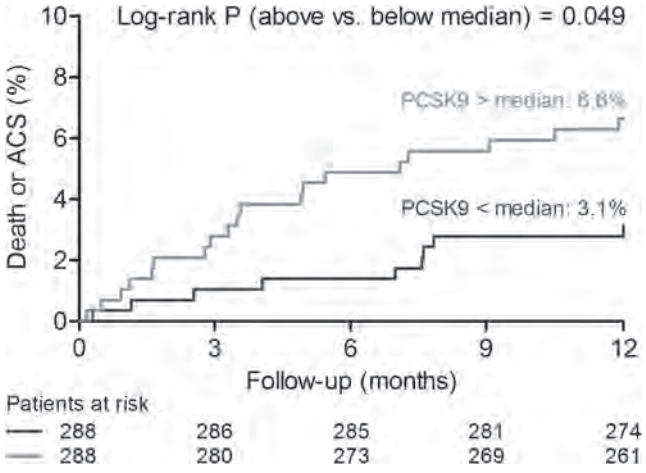

Figure 4. Association of PCSK9 level with clinical outcome
Definite culprit lesion-related events were not counted as endpoint.
ACS = acute coronary syndrome.

Table 4. Association between serum PCSK9 level and cardiovascular outcome

	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
MACE †	HR 1.11 (0.83-1.47)	0.50	HR 1.23 (0.91-1.66)	0.19	HR 1.23 (0.91-1.67)	0.18
Composite of death or ACS †	HR 1.27 (0.90-1.78)	0.17	HR 1.43 (1.02-2.01)	0.037	HR 1.42 (1.02-1.99)	0.040

Data are presented as hazard ratios (95% confidence interval) per 100 µg/L increase in serum PCSK9 level.
* Cardiac risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking. Statin use was registered at the time of hospital admission.
† Definite culprit lesion-related events were not included as endpoints.
ACS = acute coronary syndrome; CAD = coronary artery disease; LDL = low-density lipoprotein, MACE = major adverse cardiac events; PCSK9 = proprotein convertase substilisin/kexin type 9.

CI 1.02-2.01, p=0.037). This association remained similar after additional adjustment for serum LDL level (HR 1.42 per 100 µg/L increase in serum PCSK9 level, 95% CI 1.02-1.99, p=0.040).

DISCUSSION

This study investigated the association of serum PCSK9 levels with the fraction and amount of necrotic core assessed by IVUS-VH imaging of coronary atherosclerotic plaque, as well as with 1-year clinical outcome, in patients with established CAD undergoing coronary angiography. The main finding is that higher serum PCSK9 levels are linearly associated with a higher necrotic core fraction in coronary atherosclerosis. This association was independent of serum LDL level 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. 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. The second finding is that the PCSK9 serum levels also appear to be significantly associated with the composite of death and ACS during 1 year follow-up after coronary angiography. This association was also independent of established cardiac risk factors, statin use and LDL level.

PCSK9 in cholesterol homeostasis

Serum PCSK9 levels vary between individuals.(22) 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).(22) 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.(23-26) 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.(22,27-29) Recent phase II clinical trials have reported promising results on serum LDL levels by administration of monoclonal antibodies against PCSK9.(3-9)

Direct role of PCSK9 in plaque inflammation

It has been suggested that PCSK9 may also 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 level is linearly associated with the amount of necrotic core by IVUS-VH imaging, independently of serum LDL level and in all patient subgroups (including statin users and non-statin users as well as patients with low and high LDL), supports the hypothesis that PCSK9 has a direct role in plaque inflammation.

PCSK9 level and cardiovascular outcome

In the current study, a significant association between PCSK9 and the composite endpoint of death or ACS was only found after adjustment in multivariable analysis. The lack of statistical significance in univariable analysis may be explained by negative confounding by the covariates, especially statin use (positively associated with serum PCSK9 level and negatively associated with adverse coronary events). 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 homeostasis) requires further elucidation in future research.

Study limitations

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. We may have had insufficient power to detect a significant association between serum PCSK9 levels and occurrence of MACE during follow-up.

CONCLUSIONS

In patients with established CAD, the range in serum PCSK9 level is wide. The variation in PCSK9 level is linearly associated with the fraction and amount of IVUS-VH-derived necrotic core tissue in coronary atherosclerotic plaque. These associations were independent of serum LDL cholesterol level and were observed in all patient subgroups, including statin users and non-statin users, as well as patients with low and high LDL. Furthermore, serum PCSK9 levels was associated with the composite of death or ACS during 1-year follow-up after coronary angiography, independently from established cardiac risk factors, statin use and LDL levels. 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 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.

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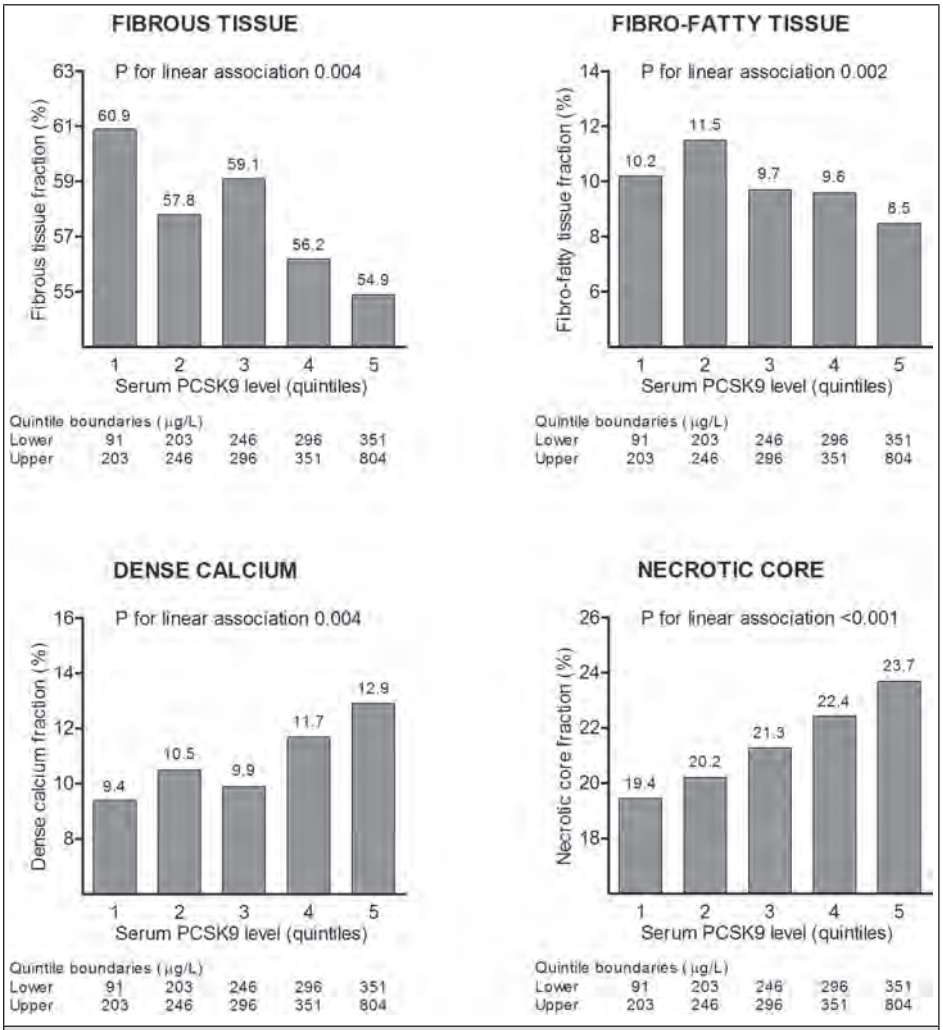
SUPPLEMENTAL FIGURES



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

Chapter 11

Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome

Cheng JM, Suoniemi M, Kardys I, Vihervaara T, De Boer, SPM, Akkerhuis KM, Sysi-Aho M, Ekroos K, Garcia-Garcia HM, Oemrawsingh RM, Regar E, Koenig W, Serruys PW, Van Geuns RJ, Boersma E, Laaksonen R.

Submitted for publication.

ABSTRACT

Rationale: Previous lipidomics analyses have demonstrated that several lipid molecules in plasma are associated with fatal outcome in patients with coronary artery disease (CAD).

Objective: This study aims to investigate the associations of previously identified high risk lipid molecules in plasma with coronary plaque characteristics derived from intravascular ultrasound virtual histology (IVUS-VH) imaging, with coronary lipid core burden index (LCBI) on near-infrared spectroscopy (NIRS), and with one year cardiovascular outcome in patients with CAD.

Methods and Results: 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 CAD. NIRS imaging was additionally performed in 191 patients. Plasma concentrations of molecular lipids were measured with mass spectrometry. Several cholesteryl ester, ceramide and lactosylceramide species and ceramide ratios were associated with vulnerable plaque characteristics on IVUS-VH and NIRS imaging and with 1-year major adverse cardiac events (MACE, defined as all-cause mortality, ACS and unplanned coronary revascularization). In particular, ceramide d18:1/16:0 was consistently associated with higher necrotic core fraction on IVUS-VH (p=0.001), higher LCBI (p=0.024) on NIRS and higher MACE rate (adjusted HR 1.79 per standard deviation increase in log-transformed lipid concentration, 95%CI 1.24-2.59, p=0.002).

Conclusions: Several molecular lipid species, and particularly ceramide(d18:1/16:0), are associated with the fraction of necrotic core tissue and lipid core burden in coronary atherosclerosis, and are predictive for 1-year clinical outcome after coronary angiography. These molecular lipids may improve risk stratification in CAD and may also be interesting therapeutic targets for the treatment of atherosclerotic disease.

INTRODUCTION

In current clinical practice, the concentration of low-density lipoprotein (LDL) cholesterol is often used for risk stratification in coronary artery disease (CAD). However, LDL cholesterol represents merely one aspect of lipid metabolism. Lipidomic analyses have demonstrated that hundreds of molecular lipid species are present in human plasma.[1] It is reasonable to assume that some of these molecular lipid species are also directly involved in the development of atherosclerosis.[2-4] Assessment of such 'high risk' molecular lipids may further improve our understanding of the development of atherosclerosis and may also improve CAD risk stratification. In fact, we have recently identified several molecular lipid species that are associated with fatal outcome in patients with CAD by performing lipidomic analysis in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.[5]

So far, lipidomics studies in CAD have mostly examined associations with clinical cardiovascular outcomes. Investigations using sophisticated imaging techniques may provide further insight into the pathophysiological role of lipid species in CAD. Intravascular ultrasound virtual histology (IVUS-VH) is an in-vivo imaging technique that analyzes radiofrequency backscatter.[6] IVUS-VH imaging allows for accurate measurement of the extent of coronary atherosclerosis and of the composition of atherosclerotic plaque, including necrotic core tissue.[6-9] Previous studies have demonstrated that the amount of necrotic core tissue on IVUS-VH predicts cardiovascular outcome.[7-9] Near-infrared spectroscopy (NIRS) is another in-vivo imaging technique that analyzes tissue scattering and absorption of light in the near-infrared wavelength region. NIRS allows for identification of plaques with lipid cores in coronary atherosclerosis.[10] We have recently demonstrated that the lipid core burden assessed by NIRS predicts cardiovascular outcome.[11]

This study aims to investigate the associations of high risk molecular lipids, previously identified in the LURIC study, with coronary plaque characteristics assessed by IVUS-VH imaging, with coronary lipid core burden assessed by NIRS imaging, and with 1 year cardiovascular outcome in patients with 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.[7,12] 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 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.

Plasma concentrations of molecular lipids

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. Stored plasma samples (n=574 patients) were subjected to lipid extraction.[13] Known amounts of internal standards were added to the samples before extraction and the final lipid extracts were dried under nitrogen. The extracts were reconstituted as described elsewhere.[13]

Sphingolipids were analyzed on a QTRAP® 5500 mass spectrometer (AB SCIEX, Concord, Canada) 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 in positive ion mode[14], using an acquity BEH C18, 2.1×50 mm column with a particle size of 1.7 µm (Waters, Milford, MA). A 25 min gradient using 10 mM ammonium acetate in water with 0.1% formic acid (mobile phase A) and 10 mM ammonium acetate in acetonitrile:isopropanol (4:3, v/v) containing 0.1% formic acid (mobile phase B) was applied. Shotgun lipidomics was performed by multiple precursor ion and neutral loss scanning on a QTRAP® 5500 mass spectrometer (AB SCIEX, Concord, Canada) equipped with a robotic nanoflow ion source NanoMate HD (Advion, NY, USA).[15]

Mass spectrometry data files were processed using MultiQuant™ 1.1.0.26 or Lipid Profiler™ 1.1 (AB SCIEX, Concord, Canada).[16] Identified lipids are quantified by normalizing against their respective internal standard and plasma volume, presented in µM. Quality control samples are utilized to monitor the overall quality of the lipid extraction and mass spectrometry analyses.[17] In 7 patients, plasma samples were not available for measurement of molecular lipid concentrations.

Molecular lipids and lipid ratios that were previously found to be associated with fatal cardiovascular outcome at a $p<0.001$ level in the Ludwigshafen Risk and Cardiovascular Health (LURIC) lipidomic study were selected for evaluation in this study.[5] These include 8 molecular lipids [cholesteryl ester (CE) 14:0, CE 18:3, CE 20:4, CE 20:5, CE 22:5, ceramide (Cer)(d18:1/16:0), Cer(d18:1/24:0), lactosylceramide (LacCer)(d18:1/18:0)] and 3 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)].

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. 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-VH data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS-VH catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second. The IVUS-VH images were sent to an independent core laboratory (Cardialysis bv, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for patient characteristics, molecular lipids and clinical outcome data. The 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. Using IVUS-VH, the composition of atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core (Figure 1).[6]

Coronary near-infrared spectroscopy imaging

A total of 191 patients also provided written informed consent for additional enrollment in the ATHEROREMO-NIRS substudy, in which NIRS imaging of the non-culprit coronary artery was performed in addition to IVUS-VH imaging.[12] The NIRS system 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.[18,19] Tissue scattering and absorption of light in the NIRS 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. 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 (Figure 1). Hence, LCBI is a summary measure of the amount of lipid core plaque along the entire imaged section of the coronary artery on a 0-1000 scale. NIRS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, The Netherlands). Core laboratory personnel are blinded to patient characteristics, molecular lipids and clinical outcome data.

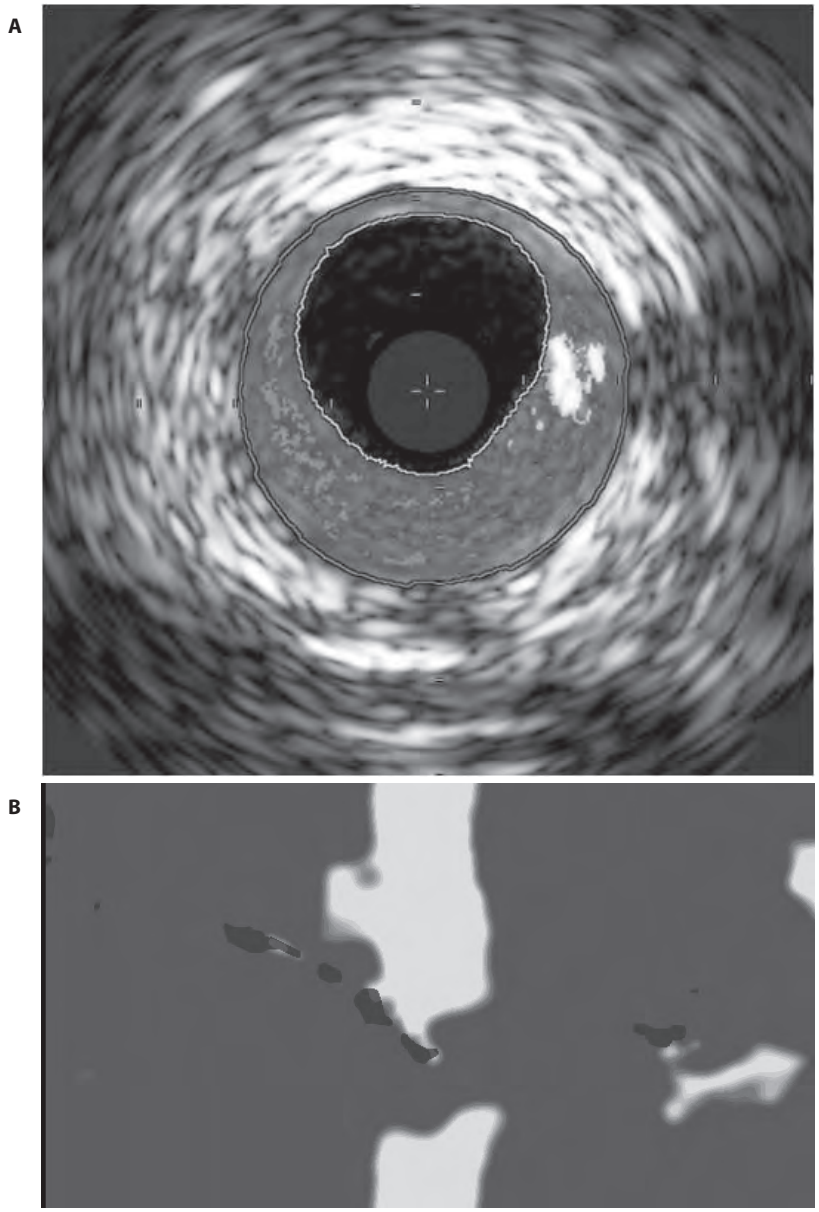


Figure 1. Coronary intravascular ultrasound virtual histology imaging and near-infrared spectroscopy

A. Intravascular ultrasound virtual histology imaging was used to characterize atherosclerotic plaque into 4 different tissue types: fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red). B. Near-infrared spectroscopy was used to compute the lipid core burden index (LCBI) score on basis of the chemogram by multiplying the fraction of valid yellow pixels within the region of interest by 1000.

See also Appendix: Color figure 2 and 5.

Follow-up and clinical endpoints

Clinical follow-up started at inclusion and lasted for 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 either as being related to the coronary site that was treated during the index procedure (culprit lesion-related event) or as being 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, the event was classified as indeterminate.

Occurrence of culprit lesion-related events is most likely caused by in-stent restenosis or in-stent thrombosis. Since, in the current study, we were specifically interested in unanticipated, spontaneous MACE, we took into account only those clinical endpoints that were defined as non-culprit lesion-related or indeterminate. These clinical endpoints were: 1. MACE, defined as non-culprit lesion-related or indeterminate mortality, ACS or unplanned coronary revascularization; and 2. the composite of non-culprit lesion-related or indeterminate mortality or ACS, both during 1 year of follow-up. Thus, definite culprit lesion-related events were not included in the clinical endpoints in this study. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction, non-ST segment elevation myocardial infarction or unstable angina pectoris. Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). The endpoints were adjudicated by a clinical event committee that had no knowledge of the molecular lipids and the IVUS-VH data.

Statistical analysis

Prior to statistical analysis the molecular lipids were log-transformed. Unpaired Student's t-test was used to evaluate the difference in mean log-transformed lipid concentration between the highest quartile and the lowest quartile of 1. plaque burden; 2. fibrous tissue percentage; 3. fibrofatty tissue percentage; 4. dense calcium percentage; 5. necrotic core percentage; and 6. LCBI. Patients lost during the 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 molecular lipids and clinical study endpoints. Age, gender, type 2 diabetes, statin use at time of hospital admission and clinical presentation (ACS or stable CAD) were a priori defined

as potential confounders, and were therefore entered as covariates in multivariate analyses. Also, baseline serum LDL cholesterol level was additionally entered into the model to evaluate whether the associations with MACE were independent of serum LDL cholesterol level. Unadjusted and adjusted hazard ratios (HR) with 95% CI were reported.

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. Since the lipids of interest were selected a priori, neither false discovery rates were estimated nor corrections for multiple hypotheses testing were applied.

RESULTS

Baseline characteristics

Mean age of the patients was 61.5 ± 11.3 years, 75% were men, and 55% were admitted with ACS (Table 1). ACS patients had higher concentrations of CE 14:0, CE 18:3, CE 22:5, Cer(d18:1/16:0), Cer(d18:1/24:0), LacCer(d18:1/18:0) and Cer(d18:1/16:0)/Cer(d18:1/24:0) than patients with stable CAD (Table 2). The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm (Table 1). Mean plaque burden on IVUS-VH imaging was 38.2 ± 11.5 percent. NIRS imaging was performed in a subgroup of 191 patients. Median LCBI on NIRS imaging was 43 [15-83].

Table 1. Baseline characteristics

		n = 574 patients
Patient characteristics	Age, years	61.5 ± 11.3
	Men, n (%)	432 (75.3)
	Diabetes Mellitus, n (%)	97 (16.9)
	Hypertension, n (%)	298 (51.9)
	Hypercholesterolemia, n (%)	318 (55.4)
	Current smoking, n (%)	166 (28.9)
	Positive family history, n (%)	298 (51.9)
	Previous MI, n (%)	184 (32.1)
	Previous PCI, n (%)	184 (32.1)
	Previous CABG, n (%)	18 (3.1)
	Previous stroke, n (%)	26 (4.5)
	History of peripheral artery disease, n (%)	35 (6.1)
	History of renal insufficiency, n (%)	32 (5.6)
	History of heart failure, n (%)	19 (3.3)
	Serum LDL cholesterol, mmol/L	2.71 [2.12-3.54]
	Statin use at time of hospital admission, n (%)	357 (62.2)

Table 1. **Baseline characteristics** (continued)		
Procedural characteristics	Indication for coronary angiography	
	ACS, n (%)	313 (54.5)
	ST-elevation MI	162 (28.2)
	Non-ST-elevation ACS	151 (26.3)
	Stable coronary artery disease, n (%)	261 (45.5)
	Number of diseased coronary vessels*	
	No significant stenosis, n (%)	42 (7.3)
	1-vessel disease, n (%)	304 (53.0)
	2-vessel disease, n (%)	167 (29.1)
	3-vessel disease, n (%)	61 (10.6)
	PCI performed, n (%)	505 (88.0)
IVUS-VH imaging	Imaged coronary artery	
	Left anterior descending, n (%)	206 (35.9)
	Left circumflex, n (%)	192 (33.4)
	Right coronary artery, n (%)	176 (30.7)
	Segment length, mm	44.1 [33.7-55.4]
	Plaque burden, %	38.2 ± 11.5
	Fibrous tissue fraction, %	57.8 ± 11.6
	Fibro-fatty tissue fraction, %	9.91 ± 6.3
	Dense calcium fraction, %	10.9 ± 7.7
	Necrotic core fraction, %	21.4 ± 8.1
NIRS imaging†	Lipid core burden index	43 [15-83]

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.

† NIRS imaging was performed in 191 patients in addition to IVUS-VH imaging.

ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; IVUS-VH, intravascular ultrasound virtual histology; LDL, low-density lipoprotein; MI, myocardial infarction; NIRS, near-infrared spectroscopy; PCI, percutaneous coronary intervention.

Association between molecular lipids and coronary plaque characteristics

Patients whose necrotic core fraction was in the highest quartile had higher concentrations of CE 18:3 (80.5 vs. 72.7 pmol/µl, p=0.040), CE 20:5 (62.8 vs. 52.4 pmol/µl, p=0.022), Cer(d18:1/16:0) (0.139 vs. 0.122 pmol/µl, p=0.001) and Cer(d18:1/24:0) (6.94 vs. 6.05 pmol/µl, p=0.003) compared to patients whose necrotic core fraction was in the lowest quartile (Table 3). Patients whose LCBI was in the highest quartile had higher concentrations of Cer(d18:1/16:0) (0.125 vs. 0.112 pmol/µl, p=0.024), Cer(d18:1/24:0) (6.76 vs. 5.42 pmol/µl, p=0.001) and LacCer(d18:1/18:0) (0.134 vs. 0.119 pmol/µl, p=0.049) compared to patients whose LCBI was in the lowest quartile. The Cer(d18:1/20:0)/Cer(d18:1/24:0) ratio was higher in patients in the highest plaque burden quartile compared to the

Table 2. Lipid concentrations

	Total (n=574)	ACS (n=313)	Stable CAD (n=261)	P
CE 14:0, pmol/µl	21.7 [15.9-28.1]	22.9 [16.5-30.5]	21.2 [15.4-26.8]	0.008
CE 18:3, pmol/µl	70.3 [51.8-90.7]	72.3 [53.6-99.5]	66.1 [50.3-85.2]	0.003
CE 20:4, pmol/µl	386 [317-457]	394 [324-453]	374 [307-471]	0.31
CE 20:5, pmol/µl	49.1 [36.3-72.6]	49.2 [36.4-72.1]	49.0 [35.9-74.7]	0.69
CE 22:5, pmol/µl	2.65 [2.00-3.62]	2.81 [2.12-3.77]	2.53 [1.90-3.40]	0.037
Cer(d18:1/16:0), pmol/µl	0.12 [0.10-0.15]	0.13 [0.11-0.17]	0.11 [0.09-0.13]	<0.001
Cer(d18:1/24:0), pmol/µl	5.98 [4.72-7.49]	6.43 [5.00-8.07]	5.65 [4.49-6.61]	<0.001
LacCer(d18:1/18:0) , pmol/µl	0.13 [0.10-0.16]	0.13 [0.11-0.16]	0.12 [0.10-0.15]	0.001
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.020 [0.018-0.024]	0.021 [0.018-0.025]	0.020 [0.017-0.023]	0.001
Cer(d18:1/20:0)/Cer(d18:1/24:0)	0.019 [0.016-0.024]	0.019 [0.015-0.024]	0.019 [0.016-0.023]	0.62
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.31 [0.26-0.36]	0.31 [0.26-0.36]	0.31 [0.26-0.36]	0.65

Concentrations are presented in µM. Data are presented as median [interquartile range]. P-value was obtained from Student's t-test for difference in log-transformed mean lipid concentration.
ACS, acute coronary syndrome; CAD, coronary artery disease.

Table 3. Associations with coronary plaque characteristics

	Plaque burden		Fibrous tissue		Fibro-fatty tissue		Dense calcium		Necrotic core		LCBI*	
	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P
CE 14:0	-14,4	0.015	0,8	0.86	-6,1	0.31	4,7	0.39	7,6	0.087	7,6	0.17
CE 18:3	-7,1	0.19	1,2	0.61	-8,0	0.17	-0,1	0.81	10,7	0.040	9,9	0.13
CE 20:4	-2,7	0.44	0,8	0.68	-5,4	0.097	1,6	0.56	4,1	0.20	9,4	0.16
CE 20:5	4,3	0.61	-0,3	0.93	-7,9	0.48	11,7	0.15	19,7	0.022	13,3	0.26
CE 22:5	-10,2	0.15	-1,1	0.80	7,7	0.27	0,9	0.83	4,4	0.13	0,5	0.84
Cer(d18:1/16:0)	-1,6	0.52	-1,8	0.48	-11,0	0.001	3,4	0.42	13,5	0.001	11,5	0.024
Cer(d18:1/24:0)	-4,6	0.15	-0,6	0.99	-11,6	0.010	-1,4	0.59	14,6	0.003	24,6	0.001
LacCer(d18:1/18:0)	4,6	0.45	-3,8	0.56	-9,9	0.029	7,3	0.30	8,4	0.14	12,7	0.049
Cer(d18:1/16:0)/ Cer(d18:1/24:0)	5,8	0.21	-3,7	0.39	-1,4	0.78	6,8	0.084	1,4	0.90	-8,9	0.039
Cer(d18:1/20:0)/ Cer(d18:1/24:0)	10,8	0.036	-8,1	0.040	-4,8	0.29	10,9	0.009	3,1	0.60	-6,4	0.27
Cer(d18:1/24:1)/ Cer(d18:1/24:0)	6,1	0.079	-3,4	0.46	0,6	0.62	8,3	0.054	-0,7	0.65	-3,2	0.47

Presented data are relative differences in mean lipid concentration between the highest quartile and the lowest quartile of 1. plaque burden; 2. fibrous tissue percentage; 3. fibrofatty tissue percentage; 4. dense calcium percentage; 5. necrotic core percentage; and 6. LCBI.
* Lipid core burden index (LCBI) was measured in 191 patients.

lowest quartile (0.0215 vs. 0.0194, p=0.036), as well as in patients in the highest dense calcium quartile compared to the lowest quartile (0.0214 vs. 0.0193, p=0.009).

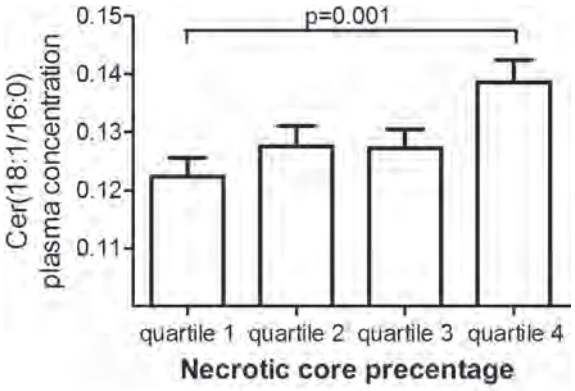
Incident major adverse cardiac events

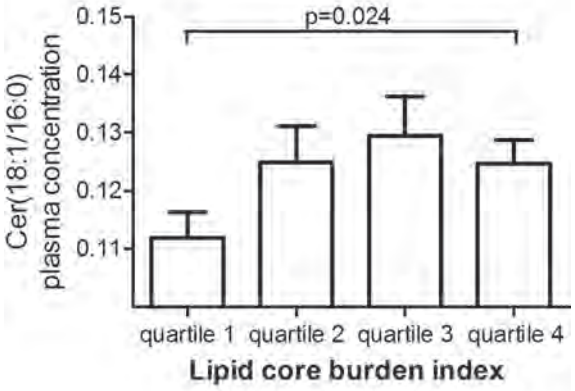
Vital status at 1-year follow-up could be acquired for 572 (99.7%) patients. Response rate of the questionnaires that were sent to all living patients was 92.0%. After 1 year of follow-up, 56 patients had experienced a MACE (Supplemental Table 1). A total of 11 patients had a definite culprit lesion 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 MACE (definite culprit lesion-related events not included as endpoint) 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 death or ACS (definite culprit lesion-related events not included) were 0.7%, 3.1%, and 4.9%, respectively.

Association between molecular lipids and cardiovascular outcome

In univariate analysis, Cer(d18:1/16:0) concentration was associated with 1-year incidence of MACE (HR 1.44 per standard deviation increase in log-transformed lipid concentration, 95%CI 1.08-1.93, p=0.014) and the composite endpoint of death or ACS (HR 1.86, 95%CI 1.29-2.69, p=0.001), as well as with the individual components of death (borderline significant, HR 1.53, 95%CI 0.97-2.42, p=0.068) and ACS (HR 2.36, 95%CI 1.32-4.21, p=0.004) (Supplemental Table 2). LacCer(d18:1/18:0) and the ratios of Cer(d18:1/16:0)/Cer(d18:1/24:0), Cer(d18:1/20:0)/Cer(d18:1/24:0) and Cer(d18:1/24:1)/Cer(d18:1/24:0) were also associated with the composite endpoint of death or ACS, but were only driven by death.

After adjustment for patient characteristics, statin use and clinical presentation in multivariate analysis, Cer(d18:1/16:0) remained significantly associated with MACE (HR 1.61, 95%CI 1.17-2.22, p=0.004) and the composite endpoint of death or ACS (HR 1.88, 95%CI 1.24-2.84, p=0.003) (Table 4). After additional adjustment for admission LDL cholesterol level, Cer(d18:1/16:0) also remained significantly associated with MACE (HR 1.79, 95%CI 1.24-2.59, p=0.002) and the composite endpoint of death or ACS (HR 2.45, 95%CI 1.55-3.87, p<0.001).





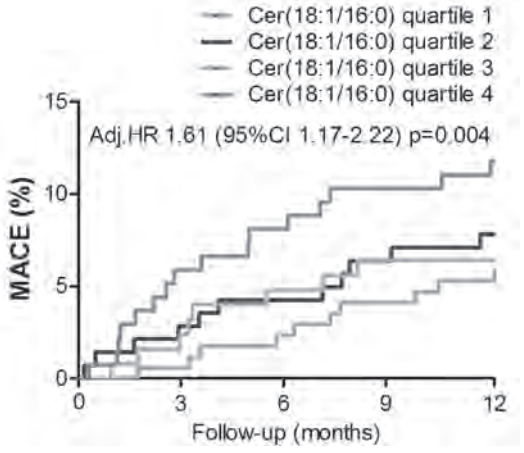


Figure 2. Association of plasma ceramide concentration with coronary plaque morphology and cardiovascular outcome

Table 4. Associations with cardiovascular outcome

	MACE		Death or ACS	
	HR (95%CI)*	P	HR (95%CI)*	P
CE 14:0	1.07 (0.77-1.47)	0.70	0.89 (0.60-1.33)	0.58
CE 18:3	1.22 (0.87-1.71)	0.26	0.97 (0.65-1.46)	0.88
CE 20:4	0.82 (0.61-1.11)	0.20	0.77 (0.53-1.11)	0.16
CE 20:5	1.22 (0.91-1.63)	0.18	1.27 (0.88-1.83)	0.20
CE 22:5	0.98 (0.73-1.32)	0.89	0.90 (0.61-1.33)	0.59
Cer(d18:1/16:0)	1.61 (1.17-2.22)	0.004	1.88 (1.24-2.84)	0.003
Cer(d18:1/24:0)	1.32 (0.95-1.83)	0.10	1.09 (0.73-1.63)	0.68
LacCer(d18:1/18:0)	1.09 (0.80-1.50)	0.58	1.23 (0.82-1.85)	0.31
Cer(d18:1/16:0)/Cer(d18:1/24:0)	1.18 (0.88-1.57)	0.28	1.56 (1.13-2.14)	0.007
Cer(d18:1/20:0)/Cer(d18:1/24:0)	1.10 (0.81-1.50)	0.54	1.54 (1.06-2.24)	0.023
Cer(d18:1/24:1)/Cer(d18:1/24:0)	1.49 (1.08-2.05)	0.014	1.84 (1.24-2.72)	0.002

Data are presented as HR per standard deviation increase in log-transformed lipid concentration. Definite culprit lesion-related events were not counted as endpoint.

* Adjusted for age, gender, diabetes, statin use and clinical presentation (ACS or stable coronary artery disease).

ACS, acute coronary syndrome; HR, hazard ratio; MACE, major adverse cardiac event.

DISCUSSION

This study investigated the association of eight previously identified high risk cholesteryl ester, ceramide and lactosylceramide lipids and three ceramide ratios with coronary plaque characteristics on IVUS-VH and NIRS imaging, as well as with 1-year clinical outcome in patients with established CAD undergoing coronary angiography. The main finding is that higher plasma concentrations of several of these molecular lipid species are associated with more vulnerable plaque morphology, reflected by a higher fraction of coronary plaque consisting of necrotic core tissue on IVUS-VH and by a higher lipid core burden on NIRS imaging. This is the first study that has demonstrated such an association for molecular lipid species circulating in plasma. Secondly, Cer(d18:1/16:0) predicts 1-year MACE after coronary angiography, while the 3 ceramide ratios predict the composite endpoint of death and ACS. These associations were independent of statin use and LDL cholesterol level.

Ceramides are a family of waxy lipid molecules and are composed of sphingosine and a fatty acid. Experimental studies have shown that ceramides and related sphingolipids are associated with the development of atherosclerosis.[5,20] Ceramides, especially lactosylceramide and glucosylceramide, accumulate in the atherosclerotic plaque,[5,21] and have been shown to suppress production of apolipoprotein E leading to an accumulation of cholesterol in macrophage foam cells.[5,22] Inhibition of the glycosphingolipid pathway

was shown to decrease atherosclerosis in mice.[5,23] Several enzymes in the ceramide synthetic pathway have been tested as potential drug targets in animal models.[5,24,25]

This study investigated eight molecular lipids and three ceramide ratios that were associated with fatal cardiovascular outcome at the $p<0.001$ level in the LURIC lipidomic study, which compared 158 CAD patients who died within 3 years of follow-up with 187 matched control patients with CAD who did not die during follow-up.[5] The following findings in this study support the hypothesis that specific ceramide molecules play an important role in development of atherosclerosis and plaque vulnerability: 1. we have found that the plasma concentration of the majority of the evaluated lipids are higher in ACS patients than in patients with stable CAD; and 2. we have found that the plasma concentration of the majority of the evaluated lipids were associated with percentage necrotic core tissue and lipid core burden as assessed by IVUS-VH and NIRS imaging, and 3. we have confirmed that Cer(d18:1/16:0) and the three evaluated ceramide ratios predict cardiovascular outcome.

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.[12] This hypothesis is supported by our previous findings that IVUS-VH and NIRS imaging in only one non-culprit vessel are associated with prognosis.[11] However, necrotic core-rich and lipid core-rich plaques elsewhere in the coronary tree (including the culprit lesion) were not assessed in our study. This may have led to an underestimation of the association between plasma lipids and vulnerable plaque morphology in the coronary tree. Secondly, in this single center study primarily designed to evaluated associations between high risk lipids and IVUS-VH the number of clinical endpoints was relatively small. Thus, we may have had insufficient power to detect significant associations between some of the molecular lipids and occurrence of MACE during follow-up.

In conclusion, plasma concentrations of several cholesteryl ester, ceramide and lactosylceramide species were associated with the fraction of necrotic core tissue on IVUS-VH imaging and with the lipid core burden on NIRS imaging of coronary atherosclerosis. Some of these molecular lipid species, and particularly ceramide(d18:1/16:0), were strongly associated with 1-year clinical outcome after coronary angiography, independently from statin use and LDL cholesterol levels. The three investigated ceramide ratios predicted the composite endpoint of death and ACS. Our results confirm the findings of previous lipidomic analysis and further supports the associations of ceramide plasma concentrations and ratios with fatal outcome by demonstrating associations with coronary plaque vulnerability. Circulating molecular lipids may potentially be used to improve risk stratification in patients with CAD and might also be interesting therapeutic targets for the treatment of atherosclerotic disease.

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SUPPLEMENTAL TABLES

Supplemental Table 1. Incidence of 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 major adverse cardiac events, 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

* Study endpoints

Supplemental table 2. Associations with cardiovascular outcome in univariate analysis

	MACE		Death or ACS		Death		ACS		PCI or CABG	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
CE 14:0	1.02 (0.76-1.38)	0.87	0.98 (0.68-1.42)	0.91	0.77 (0.49-1.19)	0.24	1.33 (0.72-2.48)	0.36	1.24 (0.78-1.99)	0.36
CE 18:3	1.04 (0.77-1.40)	0.80	0.96 (0.66-1.39)	0.82	0.63 (0.39-1.01)	0.054	1.71 (0.94-3.12)	0.078	1.34 (0.84-2.11)	0.22
CE 20:4	0.76 (0.58-1.01)	0.055	0.74 (0.52-1.04)	0.086	0.55 (0.36-0.83)	0.005	1.44 (0.78-2.68)	0.25	0.90 (0.57-1.40)	0.63
CE 20:5	1.26 (0.95-1.69)	0.11	1.39 (0.97-1.99)	0.075	1.31 (0.83-2.06)	0.25	1.35 (0.76-2.41)	0.30	1.20 (0.77-1.88)	0.42
CE 22:5	0.91 (0.68-1.23)	0.53	0.90 (0.62-1.32)	0.59	1.05 (0.66-1.67)	0.85	1.11 (0.61-2.02)	0.74	1.04 (0.66-1.65)	0.85
Cer(d18:1/16:0)	1.44 (1.08-1.93)	0.014	1.86 (1.29-2.69)	0.001	1.53 (0.97-2.42)	0.068	2.36 (1.32-4.21)	0.004	1.07 (0.68-1.67)	0.78
Cer(d18:1/24:0)	1.15 (0.85-1.54)	0.36	1.09 (0.75-1.58)	0.65	0.69 (0.44-1.08)	0.10	2.33 (1.30-4.19)	0.005	1.39 (0.88-2.18)	0.16
LacCer(d18:1/18:0)	1.17 (0.87-1.57)	0.30	1.45 (1.01-2.08)	0.046	1.60 (1.03-2.50)	0.038	1.20 (0.67-2.17)	0.54	0.89 (0.56-1.40)	0.61
Cer(d18:1/16:0)/Cer(d18:1/24:0)	1.28 (0.98-1.68)	0.075	1.73 (1.28-2.32)	<0.001	2.23 (1.60-3.10)	<0.001	0.87 (0.47-1.62)	0.67	0.65 (0.40-1.06)	0.082
Cer(d18:1/20:0)/Cer(d18:1/24:0)	1.18 (0.88-1.58)	0.28	1.65 (1.14-2.39)	0.008	2.08 (1.32-3.28)	0.002	1.16 (0.64-2.10)	0.64	0.70 (0.45-1.08)	0.11
Cer(d18:1/24:1)/Cer(d18:1/24:0)	1.64 (1.22-2.22)	0.001	2.11 (1.45-3.06)	<0.001	3.29 (2.11-5.14)	<0.001	1.03 (0.56-1.86)	0.94	1.09 (0.69-1.73)	0.71

Data are presented as HR per standard deviation increase in log-transformed lipid concentration. Definite culprit lesion-related events were not counted as endpoint.
ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; HR, hazard ratio; MACE, major adverse cardiac event; PCI, percutaneous coronary intervention.

Chapter 12

Von Willebrand Factor in relation to coronary plaque burden and presence of high risk lesions on intravascular ultrasound and cardiovascular outcome

Sonneveld MAH*, Cheng JM*, Oemrawsingh RM, De Maat MPM, Kardys I, Garcia-Garcia HM, Van Geuns RJ, Regar E, Serruys PW, Boersma E, Akkerhuis KM, Leebeek FWG.

* These authors contributed equally.

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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).

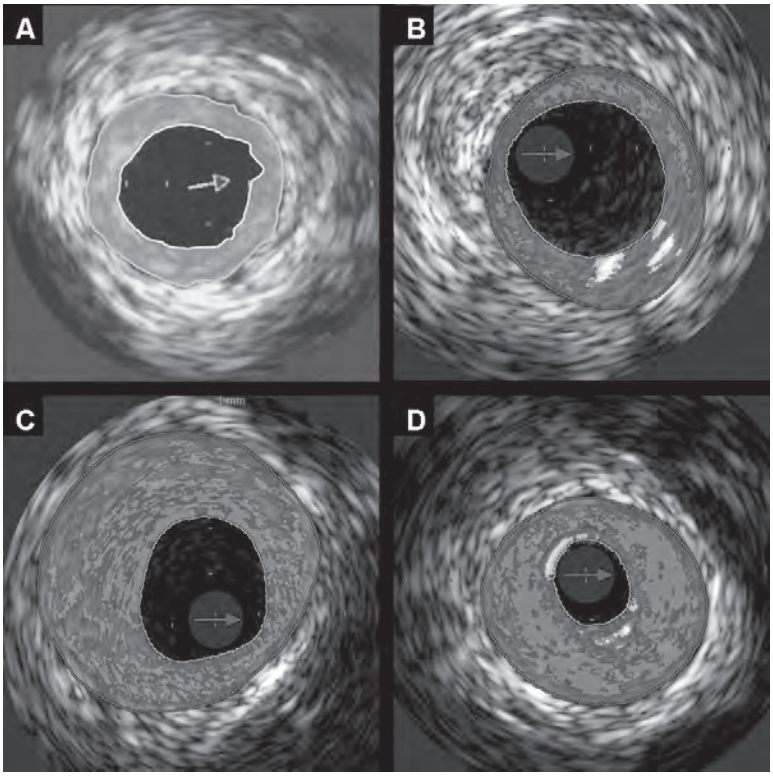

Figure 1. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound virtual histology
A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma 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.
See also Appendix: Color figure 3.

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

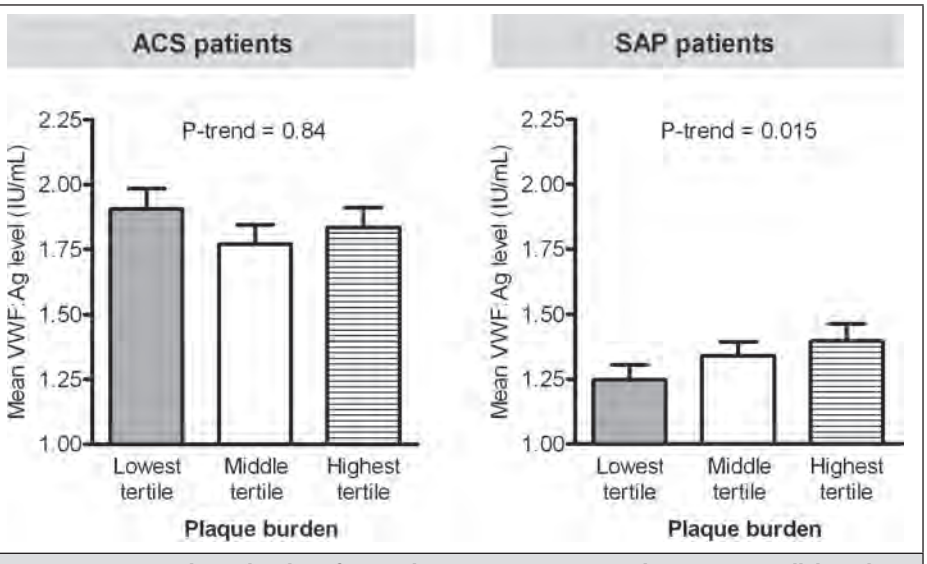

Figure 2. Coronary plaque burden of imaged coronary segment in relation to Von Willebrand Factor levels
Mean ± standard error VWF:Ag levels per tertile coronary plaque burden.
ACS = acute coronary syndrome; SAP = stable angina pectoris; VWF:Ag = von Willebrand Factor antigen.

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).

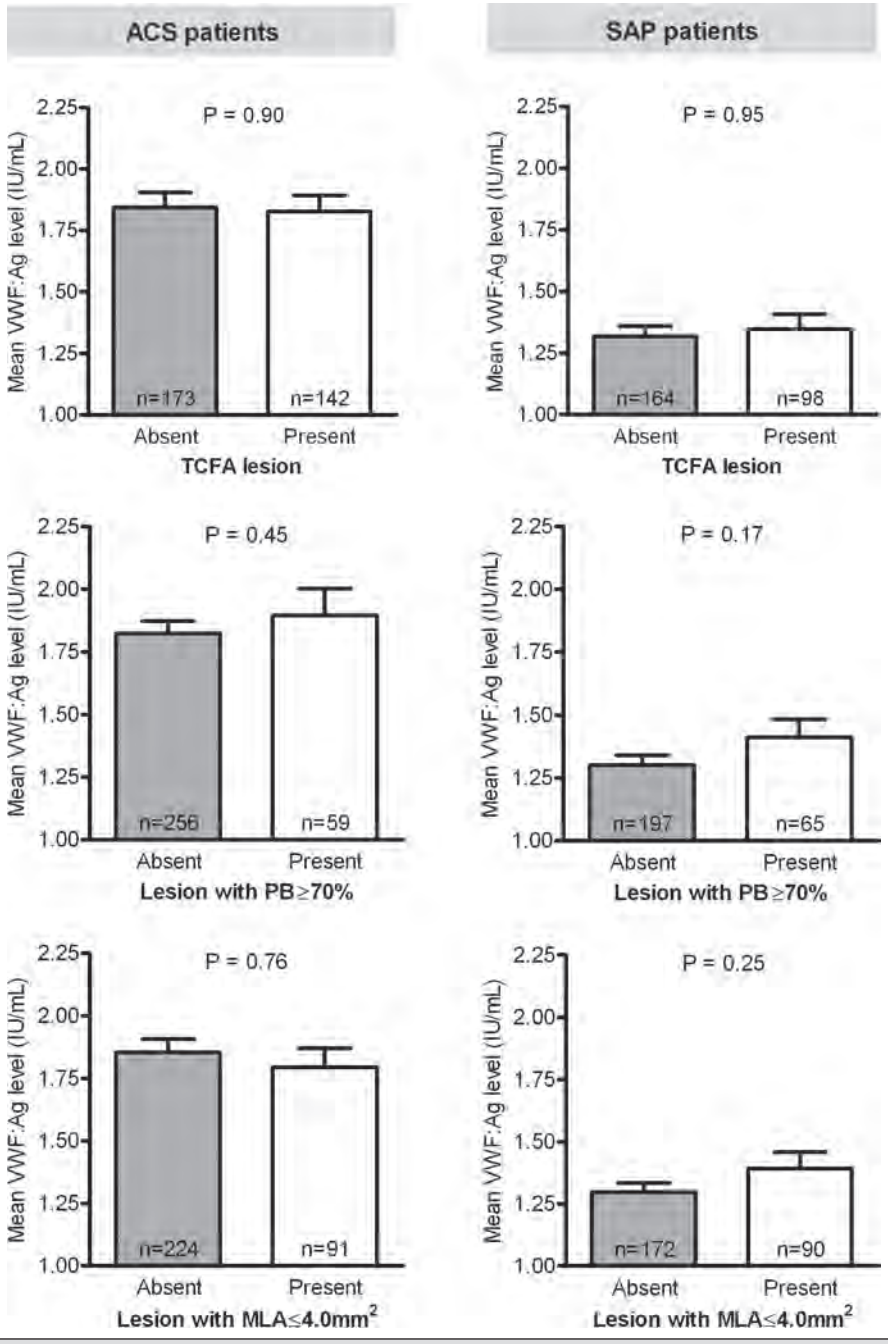

Figure 3. High risk coronary lesions in relation to Von Willebrand Factor levels
Mean ± standard error VWF:Ag levels between high-risk coronary lesions present or absent.
ACS = acute coronary syndrome; MLA = minimal luminal area; PB = plaque burden; SAP = stable angina pectoris; TCFA = thin-cap fibroatheroma; VWF:Ag = von Willebrand Factor antigen.

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.

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 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.

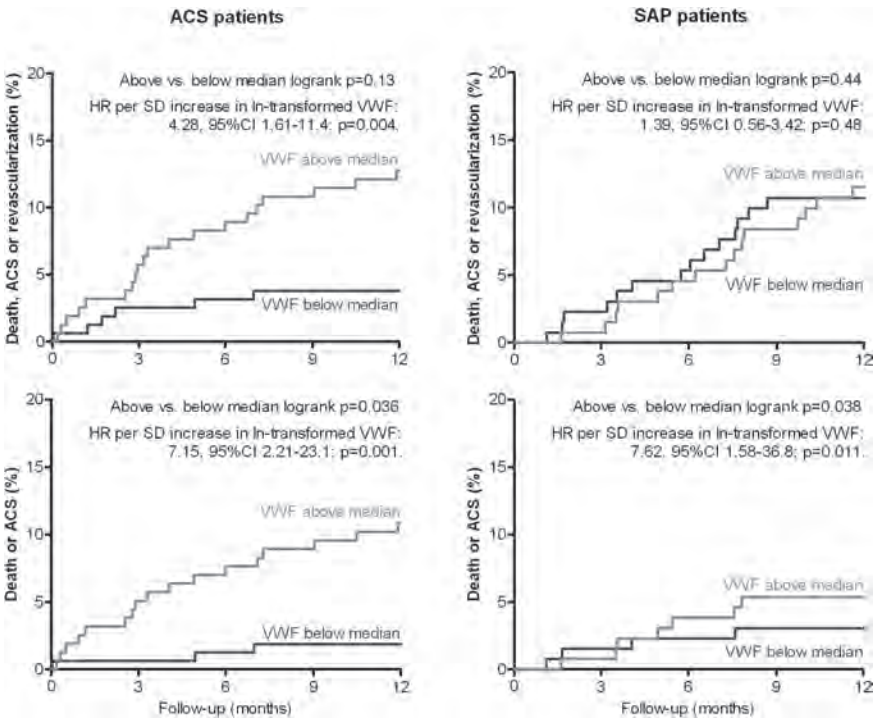

Figure 4. Von Willebrand Factor and cardiovascular outcome
Kaplan-meier curve for the cumulative event-free survival of MACE or death and hospitalization for ACS per VWF:Ag above and below the median (1.45 IU/ml).
ACS = acute coronary syndrome; SAP = stable angina pectoris.

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 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 is 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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PART III

PROGNOSTIC BIOMARKERS IN CORONARY ARTERY DISEASE

Chapter 13

High-sensitivity C-reactive protein predicts 10-year cardiovascular outcome after percutaneous coronary intervention

Oemrawsingh RM*, Cheng JM*, Akkerhuis KM, Kardys I, Degertekin M, Van Geuns RJ, Daemen J, Boersma E, Serruys PW, Van Domburg RT.

* These authors contributed equally.

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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]	

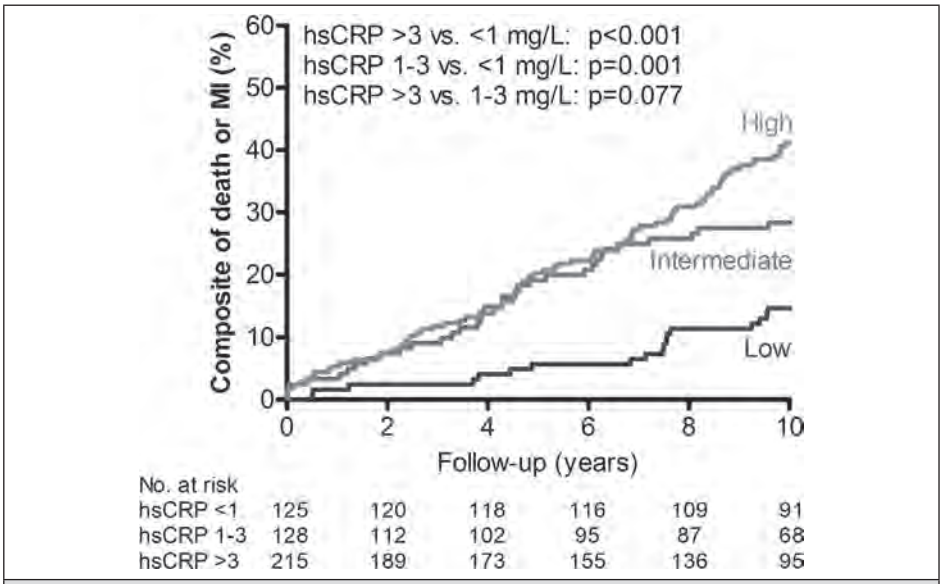

Figure 1. Prediction of cardiovascular outcome
CRP indicates C-reactive protein.

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).

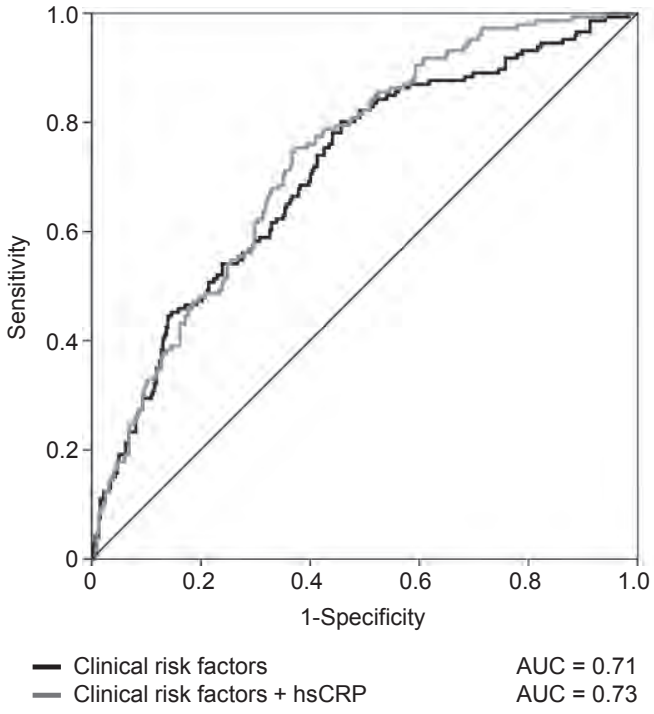

Figure 2. ROC curves displaying improved discrimination with C-reactive protein
Clinical risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, history of myocardial infarction, clinical presentation and multivessel coronary disease.
AUC indicates area under 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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Chapter 14

Circulating osteoglycin and NGAL/MMP9 complex concentrations predict 1-year major adverse cardiovascular events after coronary angiography

Cheng JM, Akkerhuis KM, Meilhac O, Oemrawsingh RM, Garcia-Garcia HM, Van Geuns RJ, Piquer D, Merle D, du Paty E, Galéa P, Jaisser F, Rossignol P, Serruys PW, Boersma E, Fareh J, Kardys I.

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ABSTRACT

Objective: Previous proteomics experiments have demonstrated that several proteins are differentially expressed in vulnerable human carotid plaques compared to stable plaques. This study aims to investigate the prognostic value of 13 of such circulating biomarkers in patients with coronary artery disease.

Approach and Results: Between 2008 and 2011, 768 patients who underwent coronary angiography for acute coronary syndrome or stable angina pectoris were included in a prospective biomarker study. Plasma concentrations of 13 biomarkers were measured in 88 patients who experienced a major adverse cardiovascular event (MACE) within 1 year, and 176 control patients without MACE who were matched on age, sex and number of diseased coronary vessels. MACE comprised all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke. After adjustment for established cardiovascular risk factors, osteoglycin (OGN; OR per SD increase in ln-transformed OGN 1.53, 95%CI 1.11-2.11, p=0.010) and neutrophil gelatinase-associated lipocalin/matrix metalloproteinase 9 (NGAL/MMP9; OR per SD increase in ln-transformed NGAL/MMP9 1.37, 95%CI 1.01-1.85, p=0.042) complex were independently associated with MACE during follow-up. These associations were independent of C-reactive protein levels. Adding OGN or NGAL/MMP9 to a model containing conventional risk factors did not significantly improve discriminatory power (OGN: area under ROC curve 0.75 vs. 0.67; NGAL/MMP9: 0.73 vs. 0.67), but did significantly improve risk reclassification (OGN net reclassification index (NRI)=0.29, 95%CI 0.05-0.53, p<0.019; NGAL/MMP9 NRI=0.44, 95%CI 0.20-0.69, p<0.001).

Conclusions: Circulating OGN and NGAL/MMP9 complex are promising biomarkers that are expressed in vulnerable atherosclerotic plaques and may have incremental value for prediction of MACE within 1 year after coronary angiography.

INTRODUCTION

Atherosclerosis and plaque destabilisation leading to coronary thrombosis and acute coronary syndrome (ACS) are the result of a heterogeneous process, involving vascular inflammation, endothelial dysfunction and hypercoagulability.[1,2] Blood biomarkers may reflect these pathophysiological constituents of coronary artery disease (CAD). Consequently, blood biomarker level may be associated with severity of CAD and thus predict occurrence of adverse cardiovascular events in CAD patients. Such associations have been demonstrated already for several biomarkers, such as C-reactive protein (CRP).[3-5]

The main challenge in investigating which biomarkers are suitable for prediction of adverse cardiovascular events is that plasma contains more than 900,000 proteins.[6] A disadvantage of traditional research is that it tests each of the plasma proteins individually. Proteomics-based research has the potential to offer insight into the full complexity of atherosclerosis with its various components and may reveal novel biomarkers that have the ability to improve prediction of adverse events.[7] In a previous proteomic study, we have found several proteins to be differentially released by vulnerable hemorrhagic human carotid plaques when compared to stable fibrotic plaques.[8] The majority of these proteins, including aciculin, oncogene DJ1, microfibril-associated glycoprotein 4 (MFAP4), osteoglycin (OGN), procollagen C proteinase enhancer 1 (PCPE1), phosphatidylethanol-amine-binding protein 1 (PEBP1) and peroxiredoxin 2 (PRX2), have not yet been investigated as prognostic biomarkers in CAD patients. Additionally, evidence exists that neutrophil gelatinase-associated lipocalin (NGAL) and its NGAL/matrix metalloproteinase 9 (NGAL/MMP9) complex display increased expression in (unstable) atherosclerotic plaques.[9,10] These proteins have also not yet been investigated as prognostic biomarkers in CAD patients.

We have performed a prospective, nested case-control study in a cohort of 768 patients undergoing coronary angiography to investigate whether plasma levels of the above-described novel protein biomarkers are associated with adverse cardiovascular events. We have also evaluated whether these biomarkers improve discrimination and risk reclassification.

METHODS

Study population and baseline data collection

Between November 2008 and January 2011, a cohort of 768 patient who underwent coronary angiography for acute coronary syndrome or stable angina pectoris in Erasmus MC, Rotterdam, the Netherlands were included in a prospective biomarker study. The current nested case-control study included a total of 88 patients that experienced

a major adverse cardiovascular event (MACE) within 1 year after the initial coronary angiography (cases), and 176 control patients who did not experience MACE and were matched on age, sex and number of diseased coronary vessels. MACE was defined as all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke. This study was approved by the medical ethics committee of Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all patients.

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

Biomarkers were chosen based on a previous discovery proteomics study that identified several proteins to be differentially released by vulnerable hemorrhagic human carotid plaques when compared to stable fibrotic plaques.[8] In the current study, ELISA assays were used to measure the biomarker concentrations.

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at a temperature of -80°C within 2 hours after blood collection.

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). 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.

Frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of -80°C) to Sysdiag Laboratory at Montpellier, where the concentrations of the 13 novel biomarkers were measured in a blinded manner. Laboratory investigators had no access to the clinical data. Using commercially available enzyme-linked immunosorbent assays (ELISA), PCPE1 (USCN Life Science, Wuhan, Hubei, China), DJ1 (Circulex Human DJ-1/PARK7 ELISA Kit, MBL International Corporation, Woburn, Massachusetts, USA), heat shock protein 27 (HSP27; Merck, Whitehouse Station, New Jersey, USA), OGN (USCN Life Science, Wuhan, Hubei, China), PRX2 (R&D Systems, Minneapolis,

Minnesota, USA), metalloproteinase inhibitor 1 (TIMP-1; R&D Systems, Minneapolis, Minnesota, USA) and thrombospondin-2 (TSP2; R&D Systems, Minneapolis, Minnesota, USA) were measured according to manufacturer's instructions. Pro-B-type natriuretic protein 1-108 (proBNP), aciculin, MFAP4, NGAL and NGAL/MMP9 were measured by home-made ELISA assays using specific and sensitive monoclonal antibodies that were developed by the HTMAb platform (Sysdiag Laboratory, Montpellier, France). Details of the assay performances have been described elsewhere.[8,11]

Clinical follow-up

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 MACE were sent to all living patients. Response rate of the questionnaires was 99.6%. 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] Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). Stroke was defined according to the guidelines of the European Stroke Organization.[13] The endpoints were adjudicated by a clinical event committee that had no knowledge of the biomarker data.

Statistical analysis

The distributions of continuous baseline characteristics, as well as the biomarker levels, were tested for normality using the Kolmogorov-Smirnov test. Variables that were not normally distributed were ln-transformed for further analyses. We used conditional logistic regression analyses to compare baseline characteristics between cases and controls. Conditional logistic regression analyses were also performed to evaluate the associations between biomarker levels and incident MACE. In multivariable analyses, the following clinical covariates were additionally entered into the model: diabetes mellitus, hypertension, smoking, dyslipidemia, history of myocardial infarction, history of renal impairment and indication for coronary angiography. Subsequently, 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 crude and adjusted odds ratios (OR) with 95% confidence interval (95% CI).

For biomarkers that were independently associated with incident MACE, 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. Furthermore, continuous net reclassification improvement indices (NRI) were calculated to evaluate improvement in risk classification by the new biomarkers over conventional cardiovascular risk factors.[14] In brief, NRI is calculated by examining cases and controls separately. It is desirable to increase the predicted probabilities of event for those who experience events, and hence any upward reclassification among events is considered beneficial. For control patients, the reasoning is reversed: downward movement in categories is considered beneficial. The total NRI is calculated as the sum of the two. All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). ROC curves were compared using the method that was described by Hanley et al.[15] All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Mean age of the patients was 64.9 (standard deviation [SD] 10.6) years, 77% were men, and 52% had ACS (Table 1). Percutaneous coronary intervention was performed in 82% of the patients. The group of patients who experienced MACE during follow-up displayed a higher prevalence of diabetes mellitus (27.3% vs. 14.9%, p=0.016) and a tendency toward a higher prevalence of renal insufficiency (11.4% vs. 4.6%, p=0.053) compared to the group of patients who did not had MACE. Other baseline characteristics did not display significant differences between cases and controls.

Associations with cardiovascular outcome

Plasma NGAL appeared to be normally distributed. Aciculin, DJ1, HSP27, MFAP4, NGAL/MMP9, OGN, PCPE1, PEBP1, proBNP, PRX2, TIMP1 and TSP2 were not normally distributed and were thus ln-transformed for further analyses. Patients with MACE during follow-up had higher NGAL (cases: median 114 [IQR 67-167] vs. controls: 96 [58-133] ng/mL, p=0.038), OGN (cases: 13.7 [8.0-18.4] vs. controls: 9.6 [7.2-13.7] ng/mL, p=0.011) and TIMP1 (cases: 147 [113-175] vs. controls: 123 [94-158] ng/mL, p=0.020) levels at the time of coronary angiography compared to patients without MACE during follow-up (Table 2). Furthermore, patients with MACE during follow-up also tended to have higher TSP2 (cases: 37.4 [30.4-48.7] vs. controls: 34.8 [28.9-42.4] ng/mL, p=0.066), proBNP (cases: 63 [16-170] vs. controls: 50 [15-123] ng/ml, p=0.088) and NGAL/MMP9 (cases: 0.43 [0.27-0.61] vs. controls: 0.35 [0.26-0.56], p=0.070) levels than patients without MACE.

After adjustment for conventional cardiovascular risk factors in multivariable conditional logistic regression analyses, higher OGN (OR per SD increase in ln-transformed OGN 1.53, 95% CI 1.11-2.11, p=0.010) and NGAL/MMP9 (OR per SD increase in ln-

Table 1. Baseline characteristics

	Total (n=264)	Cases* (n=88)	Controls** (n=176)	P
Patient characteristics				
Age, years ± SD	64.9 ± 10.6	65.8 ± 11.2	64.5 ± 10.3	MV
Men, n (%)	204 (77.3)	68 (77.3)	136 (77.3)	MV
Diabetes mellitus, n (%)	50 (19.0)	24 (27.3)	26 (14.9)	0.016
Hypertension, n (%)	154 (58.8)	52 (59.1)	102 (58.6)	0.96
Hypercholesterolemia, n (%)	155 (59.2)	52 (59.1)	103 (59.2)	0.97
Smoking, n (%)	53 (20.2)	17 (19.5)	36 (20.5)	0.81
Positive family history, n (%)	130 (49.4)	46 (52.3)	84 (48.0)	0.52
Previous MI, n (%)	105 (39.8)	38 (43.2)	67 (38.1)	0.42
Previous PCI, n (%)	95 (36.0)	37 (42.0)	58 (33.0)	0.14
Previous CABG, n (%)	16 (6.1)	7 (8.0)	9 (5.1)	0.35
Previous stroke, n (%)	25 (9.5)	9 (10.2)	16 (9.1)	0.77
Peripheral artery disease, n (%)	27 (10.3)	11 (12.5)	16 (9.1)	0.39
History of renal insufficiency, n (%)	18 (6.8)	10 (11.4)	8 (4.6)	0.053
History of heart failure, n (%)	10 (3.8)	5 (5.7)	5 (2.8)	0.27
C-reactive protein, mg/L	2.0 [0.8-5.8]	2.8 [1.1-8.8]	1.7 [0.7-5.6]	
Procedural characteristics				
Indication for catheterization				0.45
Acute coronary syndrome, n (%)	137 (51.9)	43 (48.9)	94 (53.4)	
Stable angina pectoris, n (%)	127 (48.1)	45 (51.1)	82 (46.6)	
Coronary artery disease				MV
No significant stenosis, n (%)	21 (8.0)	7 (8.0)	14 (8.0)	
1-vessel disease, n (%)	87 (33.0)	29 (33.0)	58 (33.0)	
2-vessel disease, n (%)	90 (34.1)	30 (34.1)	60 (34.1)	
3-vessel disease, n (%)	66 (25.0)	22 (25.0)	44 (25.0)	
PCI performed, n (%)	182 (82.4)	60 (77.9)	122 (84.7)	0.25

Data are presented as mean ± standard deviation or as median [interquartile range]. P values are obtained by using conditional logistic regression analyses.

* Cases: patients who experienced a major adverse cardiovascular event, including all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke, within the first year of follow-up

** Control patients were matched on age, sex, number of diseased coronary vessels.

CABG = coronary artery bypass grafting; MI = myocardial infarction; MV = matching variable; PCI = percutaneous coronary intervention.

transformed NGAL/MMP9 1.37, 95% CI 1.01-1.85, p=0.042) levels were independently associated with incident MACE during follow-up (Table 3, Figure 1). OGN (OR per SD increase in ln-transformed OGN 1.50, 95% CI 1.08-2.08, p=0.015) and NGAL/MMP9 (OR per SD increase in ln-transformed NGAL/MMP9 1.38, 95% CI 1.01-1.89, p=0.043) levels

Table 2. Difference in biomarker levels between cases and controls

	Cases* (n=88)	Controls** (n=176)	OR (95% CI)***	P
Aciculin, ng/mL	250 [167-347]	191 [104-337]	1.15 (0.88-1.50)	0.31
DJ1, ng/mL	99 [51-165]	100 [54-154]	1.00 (0.77-1.29)	0.97
HSP27, ng/mL	57.3 [28.3-84.9]	53.1 [31.4-79.5]	1.06 (0.81-1.39)	0.69
MFAP4, ng/mL	1647 [1256-2034]	1424 [1093-1777]	1.24 (0.96-1.60)	0.10
NGAL, ng/mL	114 [67-167]	96 [58-133]	1.33 (1.02-1.73)	0.038
NGAL/MMP9 complex, AU	0.43 [0.27-0.61]	0.35 [0.26-0.56]	1.29 (0.98-1.70)	0.070
OGN, ng/mL	13.7 [8.0-18.4]	9.6 [7.2-13.7]	1.47 (1.09-1.97)	0.011
PCPE1, ng/mL	850 [478-1401]	952 [584-1492]	0.99 (0.77-1.28)	0.94
PEBP1, ng/mL	96 [68-125]	95 [61-126]	1.05 (0.83-1.34)	0.69
ProBNP, pg/mL	63 [16-170]	50 [15-123]	1.30 (0.96-1.74)	0.088
PRX2, ng/mL	31.9 [24.2-44.7]	31.2 [22.9-41.9]	1.17 (0.90-1.52)	0.25
TIMP1, ng/mL	147 [113-175]	123 [94-158]	1.41 (1.06-1.89)	0.020
TSP2, ng/mL	37.4 [30.4-48.7]	34.8 [28.9-42.4]	1.28 (0.98-1.66)	0.066

Data are presented as median [interquartile range]. P values are obtained by using conditional logistic regression analyses.

* Cases: patients who experienced a major adverse cardiovascular event, including all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke, within the first year of follow-up

** Control patients were matched on age, sex, number of diseased coronary vessels.

*** Odds ratio per standard deviation increase in (ln-transformed) biomarker concentration.

AU = arbitrary unit.

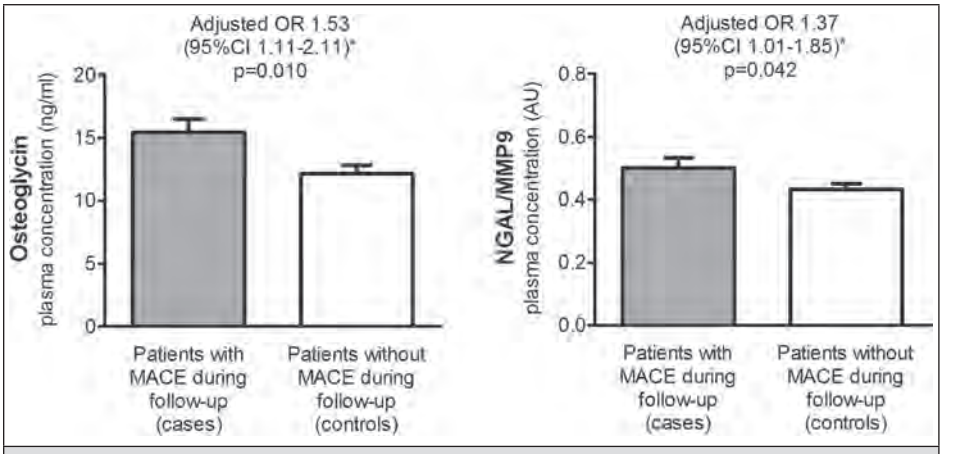

Figure 1. Associations of osteoglycin and NGAL/MMP9 complex with cardiovascular outcome

* Odds ratio per standard deviation increase in (ln-transformed) biomarker concentration. Adjusted for conventional cardiovascular risk factors: diabetes mellitus, hypertension, smoking, dyslipidemia, history of myocardial infarction, renal impairment and indication for coronary angiography.

Table 3. Associations between biomarkers and major adverse cardiovascular events in multivariable analysis

	OR (95% CI) adjusted for conventional cardiovascular risk factors*	P	OR (95% CI) adjusted for conventional cardiovascular risk factors + CRP*	P
Aciculin, ng/mL	1.15 (0.87-1.54)	0.33	1.14 (0.85-1.52)	0.39
DJ1, ng/mL	1.00 (0.76-1.32)	1.00	0.92 (0.69-1.23)	0.58
HSP27, ng/mL	1.12 (0.84-1.50)	0.43	1.03 (0.76-1.40)	0.87
MFAP4, ng/mL	1.25 (0.96-1.63)	0.10	1.22 (0.93-1.60)	0.15
NGAL, ng/mL	1.30 (0.98-1.71)	0.067	1.25 (0.93-1.67)	0.14
NGAL/MMP9 complex, AU	1.37 (1.01-1.85)	0.042	1.38 (1.01-1.89)	0.043
OGN, ng/mL	1.53 (1.11-2.11)	0.010	1.50 (1.08-2.08)	0.015
PCPE1, ng/mL	1.04 (0.79-1.35)	0.80	0.96 (0.73-1.27)	0.77
PEBP1, ng/mL	1.12 (0.86-1.46)	0.39	1.10 (0.84-1.45)	0.48
ProBNP, pg/mL	1.22 (0.90-1.67)	0.20	1.15 (0.84-1.58)	0.38
PRX2, ng/mL	1.19 (0.90-1.58)	0.22	1.21 (0.90-1.64)	0.21
TIMP1, ng/mL	1.38 (1.00-1.90)	0.051	1.22 (0.87-1.69)	0.25
TSP2, ng/mL	1.21 (0.90-1.63)	0.20	1.12 (0.82-1.53)	0.47

* Odds ratio per standard deviation increase in (ln-transformed) biomarker concentration. Conventional cardiovascular risk factors: diabetes mellitus, hypertension, smoking, dyslipidemia, history of myocardial infarction, renal impairment and indication for coronary angiography.

remained independently associated with MACE after additional adjustment for baseline CRP levels.

Discrimination

First, we evaluated a model containing conventional cardiovascular risk factors, including diabetes mellitus, hypertension, history of myocardial infarction, renal impairment and indication for coronary angiography. This model displayed an area under the ROC curve of 0.67 (Figure 2). When we added CRP to this model, it displayed an area under the ROC curve of 0.71 (p=0.41). Although not statistically significant, adding OGN (area under the ROC curve = 0.75, p=0.12 compared to model with conventional factors, p=0.47 compared to model with conventional factors + CRP) or NGAL/MMP9 (area under the ROC curve = 0.73, p=0.19 compared to model with conventional factors, p=0.64 compared to model with conventional factors+ CRP) slightly improved discriminatory ability of the model.

Reclassification

We examined whether adding OGN and NGAL/MM9 to the conditional logistic regression model consisting of conventional cardiovascular risk factors and CRP level (as

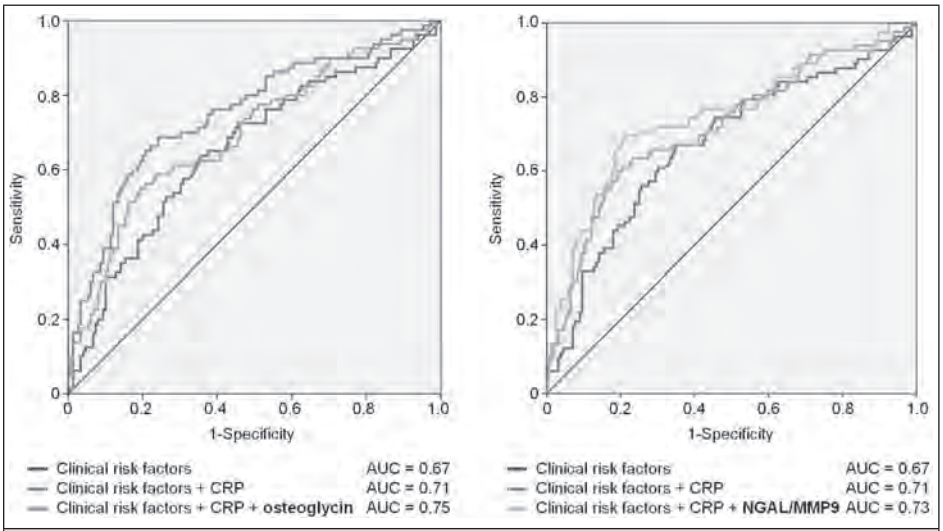

Figure 2. ROC curves displaying additional discriminatory ability of OGN and NGAL/MMP for major adverse cardiovascular events

described above and in Table 3) results in correct reclassification of risk of MACE during follow-up (Table 4). OGN (NRI = 0.29; 95% CI 0.05-0.53; p=0.019) and NGAL/MMP9 (NRI = 0.44; 95% CI 0.20-0.69; p<0.001) each significantly improved classification. Adding both OGN and NGAL/MMP9 resulted in a NRI of 0.40 (95% CI 0.17-0.64; p<0.001).

Table 4. Reclassification of predicted risk among cases and controls

	Predicted risk classified downward in new model	Predicted risk not changed in new model	Predicted risk classified upward in new model	Total
Conventional risk factors + CRP + OGN (new model) vs. risk factors + CRP only				
Cases, n (%)	30 (34.1)	8 (9.1)	50 (56.8)	88
Controls, n (%)	80 (45.5)	27 (15.3)	69 (39.2)	176
Conventional risk factors + CRP + NGAL/MMP9 (new model) vs. risk factors + CRP only				
Cases, n (%)	29 (33.0)	6 (6.8)	53 (60.2)	88
Controls, n (%)	93 (52.8)	20 (11.4)	63 (35.8)	176
Conventional risk factors + CRP + OGN + NGAL/MMP9 (new model) vs. risk factors + CRP				
Cases, n (%)	24 (27.3)	11 (12.5)	53 (60.2)	88
Controls, n (%)	78 (44.3)	33 (18.8)	65 (36.9)	176

Conventional risk factors included: diabetes mellitus, hypertension, smoking, dyslipidemia, history of myocardial infarction, history of renal impairment and indication for coronary angiography.

DISCUSSION

This study investigated the associations between circulating plasma biomarkers, which were previously identified by proteomics or immunohistochemistry experiments in human carotid plaques, and adverse cardiovascular outcome in patients undergoing coronary angiography. The prognostic value of the majority of these proteins, including OGN and NGAL/MMP9 complex, for MACE had not yet been investigated. Higher circulating OGN and NGAL/MMP9 complex levels were associated with incident MACE during the first year of follow-up, independently of conventional cardiovascular risk factors. Adding OGN or NGAL/MMP9 to a model containing conventional cardiovascular risk factors improved risk classification and discriminatory ability, although the latter was not statistically significant. These associations with incident MACE and improvements in predictive ability were independent of CRP.

In previous proteomic experiments, we have identified a series of novel potential markers of vulnerable atherosclerotic plaque.[8] We used human carotid atherosclerotic plaques, obtained from patients who underwent endarterectomy (n=80), which were classified as fibrotic plaques or hemorrhagic plaques according to the AHA classification and haemoglobin content.[8,16,17] We performed protein enrichment and mass spectrometry analysis, and subsequently validation by western blotting. We found that several tissue proteins within human atherosclerotic plaques were able to differentiate between vulnerable, hemorrhagic and stable, fibrolipidic lesions.[8] These proteins are known to be involved in various pathophysiological pathways, such as inflammation, cell integrity and arterial matrix remodeling (MFAP4, TSP2, OGN, PCPE1, TIMP1, aciculin, OGN), oxidative stress (DJ1, PRX2) and cell stress (HSP27). In the present study, we a priori hypothesized that circulating levels of these proteins may reflect presence of vulnerable plaques and may therefore also have prognostic value in patients with CAD.

Osteoglycin is a bone-associated glycoprotein, but is also found to be a basic component of the vascular extracellular matrix.[18,19] A previous study compared osteoglycin expression in atherosclerotic lesions with normal vasculature in rabbits.[18] In atherosclerotic lesions, osteoglycin was upregulated in activated endothelium and thick neointima, as well as in the front edge of migrating smooth muscle cells. Another study demonstrated that osteoglycin was substantially increased in the adventitia and neointima after balloon injury, implying a role for this protein in vessel matrix remodeling.[19] In line with these findings, close homologues of osteoglycin, such as chicken proteoglycan Lb, were found to contribute to ordering of the matrix by interacting with collagens.[19,20] Furthermore, osteoglycin has the ability to bind transforming growth factor β (TGFβ), which has been shown to have significant effects on vascular smooth muscle cell behaviour in vascular disease.[21] In our previous proteomics experiments, however, osteoglycin was found to be down-regulated in hemorrhagic plaques compared to fibrotic plaques.[8] The

lower osteoglycin levels suggested an altered strength and integrity of the vascular wall, which may make hemorrhagic plaques more prone to rupture. Its role as a prognostic circulating biomarker has also not been investigated yet. To our best knowledge, our study is the first to demonstrate that higher circulating OGN concentrations have prognostic value in patients with CAD. The seemingly contradictory results between the carotid proteomics study and the circulating levels in the present study may be due to global endothelial activation in acute conditions leading to the production of OGN, reflected by increased plasma levels. Another explanation for the decreased OGN in hemorrhagic plaques is that it may be caused by elution of OGN into the circulation. Also, differences in carotid versus coronary atherothrombotic plaque biology may provide an explanation for this discrepancy. However, evidence that may support these hypotheses is currently lacking. Again, these results underscore that the precise role of (circulating) OGN requires further investigation.

NGAL is a protein that is expressed in neutrophils, in the heart, in aorta tissue and in low levels in the kidney.[22,23] It is proposed to be a scavenger of bacterial products at sites of inflammation and a modulator of inflammation.[9] Although NGAL is well known as a biomarker of kidney injury, it has been shown that NGAL could also have a role in atherosclerotic disease.[9] NGAL is able to form a stable, biologically active complex with MMP9, preventing its degradation and thereby prolonging MMP9 activity.[16,24] MMP9 is a protease of the matrix metalloproteinase family that is capable of degrading a broad spectrum of extracellular matrix components and is held responsible for vascular remodelling and breakdown of the fibrous cap of atherosclerotic lesions leading to plaque vulnerability.[25] Several clinical studies have investigated the role of NGAL and MMP9 in cardiovascular disease, and associations of these markers with cardiovascular outcome have been found,[26,27] Our results with regard to NGAL concur with these findings. However, although NGAL was univariately associated with adverse cardiovascular events in our study, this association was not independent of conventional cardiovascular risk factors. Renal insufficiency played a part in confounding of this association (post-hoc analysis resulted in an OR adjusted for renal impairment only of 1.28 (0.97-1.68), p=0.08) The contribution of this factor was probably enhanced by the tendency toward a higher prevalence of renal insufficiency in the patients that experienced MACE.

Conversely, limited data is available on NGAL/MMP9 complex in renal insufficiency and atherosclerosis. A previous study investigated NGAL/MMP9 complex in human atherosclerotic plaques and found that increased levels of NGAL/MMP9 complex were associated with high lipid content, high number of macrophages, high interleukin-6 and interleukin-8 levels, and low smooth muscle cell content in 122 human atherosclerotic lesions.[10] These results suggest that NGAL/MMP9 complex plays a part in the inflammation cascade leading to plaque instability. To our best knowledge, our study is the first to investigate circulating NGAL/MMP9 levels in a large clinical setting and to demon-

strate that circulating NGAL/MMP9 levels have prognostic value in patients with CAD. Although NGAL/MMP9 was not significantly associated with MACE in univariate analysis (p=0.070), the association became statistically significant after adjustment for conventional risk factors (including renal insufficiency) (p=0.042). While lack of a univariable association may complicate interpretation of these findings, we believe that it does not compromise clinical utility. For the purpose of prognostication, biomarker information is traditionally evaluated in combination with the patient's clinical characteristics, and our study shows that NGAL/MMP9 significantly adds information to clinical characteristics, as demonstrated by an improvement of the NRI. MMP9 is inhibited by TIMP1, a tissue inhibitor of metalloproteinases, TIMP1 was previously found to be an independent predictor of mortality and myocardial infarction in patients with CAD.[28] We also found an association between TIMP1 and incident MACE, but in our study this association was not independent of conventional cardiovascular risk factors.

The associations of OGN and NGAL/MMP9 complex with incident MACE, and the improvements in predictive ability, were independent of CRP levels. CRP is a well known prognostic marker of cardiovascular outcome in patients with known stable CAD and patients with ACS.[3-5] CRP is produced in the liver and reflects the overall inflammatory status of the patient, [3-5] and thus does not distinguish particular causes of inflammation. Our results imply that OGN and NGAL/MMP9 may provide incremental value for prediction of cardiovascular risk on top of CRP.

The remaining biomarkers in this study did not show significant associations with MACE. Although natriuretic peptides are well known as markers for heart failure, it has been shown that natriuretic peptides also have prognostic information in patients with ACS.[29] In our study, proBNP tended to be associated with MACE in univariable analyses. We may have lacked power to detect a significant association between proBNP and MACE. With regard to HSP27, previous studies have rendered inconsistent results. With regard to TSP2, research was mainly focussed on single-nucleotide polymorphisms (SNPs). The other proteins (aciculin, DJ1, MFAP4, PCPE1, PEBP1 and PRX2) have not been investigated as prognostic biomarkers in CAD patients before. An issue that warrants consideration in our study is the fact that we examined a total of 13 biomarkers, and if we were to account for multiple testing, our findings would lose statistical significance. However, our study was not data-driven but hypothesis driven; the choice of biomarkers we investigated was based on our previous proteomics experiment. Therefore, accounting for multiple testing may not be fully justified. In any case, our findings should be considered as indicative of a potential association and merit validation in other, larger, studies.

In conclusion, OGN and NGAL/MMP9 are novel and promising prognostic biomarkers in patients with CAD. Both markers were previously found to be differentially expressed in vulnerable atherosclerotic plaques and are suggested to have important

roles in arterial matrix remodeling and endothelial activation. Higher circulating OGN and NGAL/MMP9 levels were independently predictive for occurrence of MACE within 1 year after coronary angiography, and displayed incremental value over conventional cardiovascular risk factors and CRP in terms of risk reclassification. Further studies are required to determine the precise pathophysiological role of OGN and NGAL/MMP9 in atherosclerosis, and to confirm their roles as prognostic biomarkers of CAD.

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Chapter 15

Evaluation of 42 cytokines, chemokines and growth factors for prediction of cardiovascular outcome in patients undergoing coronary angiography

Cheng JM, Akkerhuis KM, Malaud E, Piquer D, Merle D, Van Geuns RJ, Boersma E, Kardys I, Fareh J.

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Over the past decades, it has been well established that atherosclerosis is a chronic inflammatory disease.[1] Consequently, circulating biomarkers of inflammation may improve prognostication of patients with cardiovascular disease. C-reactive protein has been investigated most extensively in this context.[2] However, markers of inflammation are manifold, and studies on the predictive value of several of these markers are less abundant. The current study used a multiplex assay to identify a large series of biomarkers of inflammation that could be promising for prediction of cardiovascular outcome in patients with established coronary artery disease (CAD).

Between 2008 and 2011, a cohort of 768 patient who underwent coronary angiography for acute coronary syndrome or stable angina pectoris in Erasmus MC, Rotterdam, the Netherlands were included in a prospective biomarker study.[3] The current nested case-control study included a total of 88 patients that experienced a major adverse cardiovascular event (MACE) within 1 year after the initial coronary angiography (cases), and 176 control patients who did not experience MACE and were matched on age, sex and number of diseased coronary vessels. MACE was defined as all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke. This study was approved by the medical ethics committee of Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all patients.

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.

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at a temperature of -80 degrees Celsius within 2 hours after blood collection. A total of 42 biomarkers of inflammation, including cytokines, chemokines and growth factors, were measured in the stored EDTA-plasma samples using magnetic bead based multiplex assays (Bio-Plex Pro, Bio-Rad Laboratories, Hercules, CA, USA).

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

MACE were sent to all living patients. Response rate of the questionnaires was 99.6%. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information whenever necessary. ACS was

Table 1. Baseline characteristics

	Total (n=264)	Cases[a] (n=88)	Controls[b] (n=176)	P
Patient characteristics				
Age, years ± SD	64.9 ± 10.6	65.8 ± 11.2	64.5 ± 10.3	MV
Men, n (%)	204 (77.3)	68 (77.3)	136 (77.3)	MV
Diabetes mellitus, n (%)	50 (19.0)	24 (27.3)	26 (14.9)	0.016
Hypertension, n (%)	154 (58.8)	52 (59.1)	102 (58.6)	0.96
Hypercholesterolemia, n (%)	155 (59.2)	52 (59.1)	103 (59.2)	0.97
Smoking, n (%)	53 (20.2)	17 (19.5)	36 (20.5)	0.81
Positive family history, n (%)	130 (49.4)	46 (52.3)	84 (48.0)	0.52
Previous MI, n (%)	105 (39.8)	38 (43.2)	67 (38.1)	0.42
Previous PCI, n (%)	95 (36.0)	37 (42.0)	58 (33.0)	0.14
Previous CABG, n (%)	16 (6.1)	7 (8.0)	9 (5.1)	0.35
Previous stroke, n (%)	25 (9.5)	9 (10.2)	16 (9.1)	0.77
Peripheral artery disease, n (%)	27 (10.3)	11 (12.5)	16 (9.1)	0.39
History of renal insufficiency, n (%)	18 (6.8)	10 (11.4)	8 (4.6)	0.053
History of heart failure, n (%)	10 (3.8)	5 (5.7)	5 (2.8)	0.27
C-reactive protein, mg/L	2.0 [0.8-5.8]	2.8 [1.1-8.8]	1.7 [0.7-5.6]	
Procedural characteristics				
Indication for catheterization				0.45
Acute coronary syndrome, n (%)	137 (51.9)	43 (48.9)	94 (53.4)	
Stable angina pectoris, n (%)	127 (48.1)	45 (51.1)	82 (46.6)	
Coronary artery disease				MV
No significant stenosis, n (%)	21 (8.0)	7 (8.0)	14 (8.0)	
1-vessel disease, n (%)	87 (33.0)	29 (33.0)	58 (33.0)	
2-vessel disease, n (%)	90 (34.1)	30 (34.1)	60 (34.1)	
3-vessel disease, n (%)	66 (25.0)	22 (25.0)	44 (25.0)	
PCI performed, n (%)	182 (82.4)	60 (77.9)	122 (84.7)	0.25

Data are presented as mean ± standard deviation or as median [interquartile range]. P values are obtained by using conditional logistic regression analyses.

[a] Cases: patients who experienced a major adverse cardiovascular event, including all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke, within the first year of follow-up

[b] Control patients were matched on age, sex, number of diseased coronary vessels.

CABG = coronary artery bypass grafting; MI = myocardial infarction; MV = matching variable; PCI = percutaneous coronary intervention.

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.[4] Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). Stroke was defined according to the guidelines of the European Stroke Organization.[5] The endpoints were adjudicated by a clinical event committee that had no knowledge of the biomarker data.

The distributions of continuous baseline characteristics, as well as the biomarker levels, were tested for normality using the Kolmogorov-Smirnov test. Variables that were not normally distributed were ln-transformed for further analyses. We used conditional logistic regression analyses to compare baseline characteristics between cases and controls. Conditional logistic regression analyses were also performed to evaluate the associations between biomarker levels and incident MACE. Baseline characteristics that differed between cases and controls, as well as the indication of coronary angiography (ACS or stable CAD), were considered to be potential confounders and were entered as covariates into the multivariable analyses. The final results are presented as crude and adjusted odds ratios (OR) with 95% confidence interval (95% CI). The Bonferroni correction was applied to account for multiple testing (α=0.0012). All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed.

Mean (standard deviation) age of the patients was 64.9 (10.6) years, 77% were men, 52% of the patients were admitted with ACS and 48% of the patients had stable CAD (Table 1). The cases had a higher prevalence of diabetes (27.3% vs. 14.9%, p=0.016) and a tendency toward a higher prevalence of renal insufficiency (11.4% vs. 4.6%, p=0.053) than controls. Other baseline characteristics were similar in cases and controls.

A total of 42 biomarkers were evaluated (Table 2). After adjustment for diabetes, renal insufficiency and indication of coronary angiography (on top of the matching for age, gender and number of diseased coronary vessels), levels of interleukin-18 (IL18, OR 1.42, 95%CI 1.06-1.88, p=0.017), C-X-C-motif ligand-8 (CXCL8, OR 1.42, 95%CI 1.07-1.89, p=0.015) and C-X-C-motif ligand-9 (CXCL9, OR 1.51, 95%CI 1.12-2.04, p=0.007) were positively associated with MACE at the p=0.05 level. However, none of these associations remained statistically significant after Bonferroni correction (i.e. none of the associations reached a p-value <0.0012).

IL18, CXCL8 (also known as IL8) and CXCL9 (also known as MIG, Monokine Induced by interferon-Gamma) are known to promote inflammatory reactions in atherosclerosis by activating interferon-γ and by attracting neutrophils and T-cells, respectively.[6-8] Although clinical studies on these markers are currently still scarce, some of them point towards potential associations between circulating levels of these markers and cardiovascular outcome. For example, IL18 was previously found to be associated with

Table 2. Association between biomarkers and major adverse cardiac events

	Normally distributed	Cases[a] (n=88) Median [IQR]	Controls[b] (n=176) Median [IQR]	Adjusted OR (95% CI)[c]	P
Cytokines					
IFNa2	no	592 [492-646]	564 [497-610]	1.25 (0.94-1.67)	0.13
IFNg	no	79.7 [52.3-107.2]	72.9 [45.2-97.9]	1.16 (0.89-1.52)	0.27
IL1b	no	2.05 [1.36-3.03]	1.88 [1.26-2.63]	1.17 (0.90-1.53)	0.25
IL1Ra	no	334 [224-460]	288 [190-427]	1.22 (0.93-1.59)	0.15
IL2	no	4.10 [1.69-6.74]	3.25 [0.64-6.38]	1.15 (0.87-1.51)	0.34
IL2Ra	yes	897 [745-1012]	849 [736-963]	1.06 (0.81-1.41)	0.66
IL3	no	1548 [1093-1901]	1449 [1138-1787]	1.01 (0.78-1.32)	0.92
IL4	no	5.73 [3.54-6.96]	4.73 [3.15-7.14]	1.17 (0.89-1.53)	0.26
IL5	no	5.68 [3.48-8.52]	5.18 [3.24-7.20]	1.25 (0.95-1.66)	0.11
IL6	no	14.8 [10.4-22.3]	13.1 [8.9-17.6]	1.29 (0.98-1.70)	0.075
IL7	no	11.2 [8.6-14.8]	9.7 [6.6-13.3]	1.30 (0.99-1.71)	0.061
IL9	no	26.2 [18.4-41.0]	25.4 [16.3-40.2]	1.08 (0.83-1.41)	0.55
IL10	no	4.08 [2.69-6.63]	3.71 [2.36-6.17]	1.31 (0.98-1.76)	0.066
IL12	no	16.9 [11.4-25.5]	13.8 [9.5-20.9]	1.25 (0.95-1.65)	0.12
IL13	no	5.50 [3.65-9.03]	5.81 [3.81-8.65]	0.89 (0.67-1.19)	0.43
IL16	yes	1396 [1204-1563]	1356 [1193-1555]	1.17 (0.89-1.55)	0.27
IL17	no	21.1 [11.0-30.7]	17.7 [9.7-32.0]	1.09 (0.83-1.43)	0.52
IL18	no	756 [626-852]	692 [602-778]	1.42 (1.06-1.88)	0.017
MIF	yes	16449 [14258-19439]	16068 [13703-18965]	1.14 (0.86-1.50)	0.37
TNF	no	25.8 [13.2-42.6]	24.5 [10.4-37.2]	1.08 (0.82-1.43)	0.57
TRAIL	yes	2116 [1752-2577]	2086 [1799-2481]	1.23 (0.92-1.64)	0.16
Chemokines					
CCL2	no	21.6 [15.3-28.8]	22.9 [16.3-34.4]	0.97 (0.75-1.26)	0.83
CCL3	no	5.73 [3.73-8.19]	4.94 [3.40-7.08]	1.19 (0.91-1.57)	0.21
CCL4	no	51.8 [40.1-75.6]	54.1 [39.9-74.2]	1.00 (0.77-1.31)	0.98
CCL5	no	2645 [2131-3610]	2836 [2083-3940]	1.06 (0.81-1.39)	0.67
CCL7	yes	317 [222-429]	306 [240-369]	1.13 (0.85-1.50)	0.40
CCL11	no	45.0 [29.9-68.8]	40.3 [29.9-70.5]	1.05 (0.81-1.36)	0.73
CCL27	yes	2815 [2534-3144]	2724 [2360-3094]	1.28 (0.93-1.76)	0.13
CXCL1	no	858 [637-1068]	795 [596-1034]	1.15 (0.86-1.54)	0.35
CXCL8	no	14.6 [11.0-18.7]	12.3 [8.9-15.3]	1.42 (1.07-1.89)	0.015
CXCL9	no	2326 [1827-2711]	2070 [1574-2585]	1.51 (1.12-2.04)	0.007
CXCL10	no	384 [256-804]	337 [216-649]	1.28 (0.98-1.67)	0.076
CXCL12	yes	2049 [1764-2411]	2000 [1761-2266]	1.07 (0.82-1.41)	0.62
Growth factors					
bNGF	yes	315 [244-368]	291 [227-345]	1.21 (0.92-1.59)	0.17
FGFbasic	no	14.8 [7.1-24.0]	14.5 [6.9-21.1]	1.06 (0.81-1.38)	0.67

Table 2. Association between biomarkers and major adverse cardiac events (continued)

	Normally distributed	Cases[a] (n=88) Median [IQR]	Controls[b] (n=176) Median [IQR]	Adjusted OR (95% CI)[c]	P
GCSF	yes	180 [125-229]	155 [100-218]	1.23 (0.94-1.60)	0.13
HGF	no	1913 [1183-5351]	1363 [1142-5643]	1.27 (0.93-1.73)	0.14
MCSF	no	613 [395-837]	558 [419-842]	0.98 (0.74-1.30)	0.87
PDGFbb	no	945 [487-1724]	911 [439-1667]	1.12 (0.84-1.49)	0.44
SCF	yes	1621 [1335-1810]	1508 [1273-1770]	1.09 (0.82-1.45)	0.55
SCGFb	no	42604 [35380-47986]	39604 [33605-48123]	1.30 (0.97-1.75)	0.077
VEGF	no	24.9 [14.2-38.3]	22.1 [13.5-34.3]	1.09 (0.84-1.42)	0.52

Plasma concentrations (median [interquartile range]) are presented in pg/mL.

[a] All patients who experienced a major adverse cardiovascular event, including all-cause mortality, acute coronary syndrome, unplanned coronary revascularization and stroke, within one year after baseline coronary angiography were defined as cases.

[b] Control patients were matched on age, sex, number of diseased coronary vessels.

[c] Odds ratio per standard deviation increase in (ln-transformed) biomarker concentration. Adjusted for diabetes, renal insufficiency and indication of coronary angiography (ACS or stable CAD).

bNGF = β-nerve growth factor; CCL = C-C motif ligand; CXCL = C-X-C motif ligand; FGFbasic = fibroblast growth factor-basic; GCSF = granulocyte colony-stimulating factor; HGF = hepatocyte growth factor; IFN = interferon; IL = interleukin; MCSF = macrophage colony-stimulating factor; MIF = macrophage migration inhibitory factor; PDGFbb = platelet derived growth factor BB chain; SCF = stem cell factor; SCGFb = stem cell growth factor beta; TNF = tumor necrosis factor; TRAIL = TNF-related apoptosis-inducing ligand; VEGF = vascular endothelial growth factor.

restenosis after percutaneous coronary intervention.[9] Another study demonstrated that CXCL8 in patients with established CAD is associated with occurrence of MACE during a follow-up period of 7 years.[10]

In conclusion, 3 out of the 42 biomarkers that we investigated using a multiplex assay, specifically IL18, CXCL8 and CXCL9, could be potential predictors of cardiovascular outcome in patients with established CAD. These findings merit validation in other, larger, studies.

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PART IV

MANAGEMENT AND OUTCOME OF CARDIOGENIC SHOCK

Chapter 16

A simple risk chart for initial risk assessment of 30-day mortality in patients with cardiogenic shock from ST-elevation myocardial infarction

Cheng JM*, Helming AM*, Van Vark LC, Kardys I, Den Uil CA, Jewbali LSD, Van Geuns RJ, Zijlstra F, Van Domburg RT, Boersma E, Akkerhuis KM.

* These authors contributed equally.

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ABSTRACT

Aims: Early risk stratification is important in patients with cardiogenic shock from ST-elevation myocardial infarction (STEMI). We aimed to develop a simple risk chart that includes clinical parameters that are readily available at time of hospital admission to assess risk of 30-day mortality.

Methods and Results: A series of 544 STEMI patients admitted to undergo primary PCI and presenting with cardiogenic shock were included between 2000 and 2012. Overall 30-day mortality was 38.4% and did not change over the years (p-trend=0.64). Baseline variables that were available at time of hospital admission were entered into a logistic regression model in a forward stepwise manner. Only age (OR per year 1.05, 95%CI 1.04-1.07, p<0.001), initial serum lactate level (OR per mmol/L 1.17, 95%CI 1.11-1.24, p<0.001) and initial creatinine level above the upper limit of normal (OR 2.89, 95%CI 1.90-4.37, p<0.001) remained independent predictors, and were subsequently used to develop a risk chart that stratifies risk of 30-day mortality into categories ranging from 0-20% to 80-100%. The calibration plot showed a close relationship between expected and observed mortality. The risk chart had a higher discriminative accuracy than the GRACE score (c-index 0.75 vs. 0.66, p=0.009). Adding variables that were obtained from coronary angiography and during clinical course did not significantly improve discriminative accuracy of risk chart (c-index 0.77, p=0.48).

Conclusion: Mortality of patients with cardiogenic shock from STEMI undergoing primary PCI can be well predicted already at time of hospital admission by a risk chart that uses only three variables, namely age, initial serum lactate and creatinine level.

INTRODUCTION

Cardiogenic shock complicates 6-10% of all cases of ST-segment elevation myocardial infarction (STEMI).[1] Despite improvement in therapy over the last decades, including use of primary percutaneous coronary intervention (PCI), cardiogenic shock remains the leading cause of death in patients with STEMI.[1] The in-hospital mortality rate of acute STEMI complicated by cardiogenic shock has been reported to be as high as 50%.[2]

In patients with cardiogenic shock from STEMI, early risk assessment is important for clinical decision making in the acute phase. An initial estimate of the patient's mortality risk may, for example, be used to determine the need for and timing of initiation of mechanical circulatory support, as well as to select the type of assist device to be used, such as an intra-aortic balloon pump (IABP), percutaneous left ventricular assist device (LVAD) or extracorporal membrane oxygenation (ECMO).[3] However, established risk scores that are used in acute coronary syndrome (ACS) patients in general (e.g. Global Registry of Acute Coronary Events (GRACE) score) do not include parameters of shock severity, and may therefore be less accurate in patients with cardiogenic shock.[4] On the other hand, several outcome predictors that reflect the severity of cardiogenic shock and organ failure (e.g. cardiac index and brain damage) can often not be obtained at time of hospital admission and can therefore not be used for an initial rapid risk assessment. The aim of this study is to identify clinical predictors of 30-day mortality that may be readily available at time of hospital admission, and subsequently, to develop a simple risk chart for initial risk assessment of 30-day mortality in patients with cardiogenic shock from STEMI.

METHODS

Study population

Between January 2000 and December 2012, all STEMI patients admitted to undergo primary PCI and presenting with cardiogenic shock (at time of arrival at the catheterization laboratory or developed cardiogenic shock during the procedure) at the Erasmus MC in Rotterdam, the Netherlands, were included in the present analysis. Our center is a tertiary referral center for primary PCI, mechanical circulatory support for cardiogenic shock and heart transplantation. During the inclusion period, approximately 450 STEMI patients were admitted to our center annually. STEMI was defined according to the guidelines of the European Society of Cardiology (ESC) as acute myocardial infarction with clinical symptoms of ischemia, persistent (>20 min) ST-segment elevation in at least 2 contiguous precordial leads or at least 2 adjacent limb leads by ECG, and a diagnostic rise in cardiac markers during the hospitalization period.[1] Cardiogenic shock was defined as systolic blood pressure <90 mmHg due to cardiac insufficiency with clinical signs of

hypoperfusion (cold extremities, oliguria, altered mental state etc.), not responsive to fluid resuscitation, or need for inotropic agents or mechanical support to achieve sufficient blood pressure).[1]

Ethics

This is an observational study. For the purpose of this study, patients were not subject to acts, neither was any mode of behavior imposed, otherwise than as part of their regular treatment. Therefore, according to Dutch law, written informed consent for a patient to be enrolled in this study was not required. This study was conducted according to the Privacy Policy of the Erasmus MC, and according to the Erasmus MC regulations for the appropriate use of data in patient oriented research.

Patient management

Patient management was in accordance with the STEMI guidelines of the ESC.[1,5,6] During the study period, primary PCI was the standard treatment of STEMI. Patients received aspirin and clopidogrel (300 to 600 mg) loading dose prior to primary PCI. Use of periprocedural glycoprotein IIb/IIIa antagonists was left at the discretion of the interventional cardiologist. Inotropic agents used in our hospital were catecholamines (dobutamine, dopamine, and/or norepinephrine) and phosphodiesterase inhibitors (enoximone). IABP counterpulsation was the method of first choice for mechanical circulatory assistance in the patients with cardiogenic shock who did not adequately respond to standard pharmacologic treatment.[7-9]

Data collection and follow-up

Baseline, clinical and procedural characteristics were prospectively entered into digital patient records. These data were retrospectively retrieved and recorded in a dedicated database. Initial serum lactate, creatinine and troponin T levels were measured in blood that was drawn at the emergency department as part of routine clinical practice. The reported blood pressure and heart rate were measured at arrival in the catheterization laboratory. In December 2013, vital status of the patients was acquired from municipal civil registries. Primary study endpoint was 30-day all-cause mortality.

Statistical analysis

For most variables, less than 1% of the data were missing. Data were missing in 4.8% of the study population for blood pressure (at arrival in the catheterization laboratory), 12.1% for heart rate, 7.2% for serum creatinine, 9.7% for troponin T and 33.3% for lactate. Multiple imputation was used to create 10 imputed datasets (i.e. stochastic regression, including all baseline variables, are run on the same data set for 10 times and the imputed data sets are saved for later analysis; multiple imputation was performed with SPSS

20.0, IBM corp., Armonk, NY, USA).[10-12] All analyses were performed separately for each dataset, where after the effect estimates were combined using the method described by Rubin et al.[10-12] The final prediction model was validated in the unimputed dataset by assessing the discriminative power.

Cumulative mortality was estimated according to the Kaplan-Meier method. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Kaplan-Meier curves were compared by the log-rank test. Univariable logistic regression analyses were performed to examine the individual associations between the baseline characteristics and 30-day mortality. Additional logistic regression analyses were performed to test (by eyeballing) for a linear association between continuous variables and the outcome by dividing the continuous variables into deciles. Continuous variables that had a non-linear association with the outcome were categorized for further analyses. Subsequently, a multivariable forward stepwise logistic regression approach was used to identify independent predictors of 30-day mortality. The final results are presented as unadjusted and adjusted odds ratios (OR) with the associated 95% confidence intervals (95% CI).

The independent predictors were used to develop a risk chart. Continuous variables were categorized by using cut-off values that are regularly used in clinical practice (e.g. upper limit of normal (ULN) value of blood markers). Calibration of the risk chart was evaluated by plotting the actual mortality rates versus the expected mortality rates in each risk category (quintile). The discriminative power of the risk chart was assessed by the mean of the area under the receiver operation characteristic curve (c-index), and was compared to that of the GRACE score applied in the current study population.[4] The GRACE score is the most frequently used risk score for ACS patients in general that are admitted to our hospital. Additionally, we assessed whether adding variables that were obtained from coronary angiography and clinical course during intensive cardiac care unit stay would significantly improve discriminative power of the model.

All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). Receiver operation characteristic curves were compared using the method that was described by Hanley et al.[13] All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Predictors of 30-day mortality

The mean age of the patients (n=544) was 64 ± 12 years and 75% were men (Table 1). IABP was used in 80.0% of the patients, and did not change over the years. Vital status at 30 days could be acquired for 541 (99.4%) patients. Cumulative 30-day mortality was

Table 1. Characteristics of patients with cardiogenic shock from STEMI known at time of hospital admission

	Total n=544
Age, years	63.7 ± 12.0
Men, n (%)	406 (74.6)
History of diabetes, n (%)	84 (15.4)
History of hypertension, n (%)	173 (31.8)
History of prior MI, n (%)	109 (20.0)
History of prior PCI, n (%)	50 (9.2)
History of prior CABG, n (%)	26 (4.8)
History of renal failure, n (%)	25 (4.6)
Out-of-hospital cardiac arrest, n (%)	146 (26.8)
Systolic blood pressure, mmHg	91 ± 28
Diastolic blood pressure, mmHg	60 ± 18
Mean arterial pressure, mmHg	70 ± 20
Heart rate, bpm	91 ± 27
Acute MI located anterior/lateral, n (%)	353 (64.9)
Initial serum troponin T, µg/L	0.38 [0.05-2.65]
Initial serum lactate, mmol/L	3.4 [2.0-6.8]
Initial serum creatinine, µmol/L	93 [77-117]

Presented data are not imputed.
CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention.

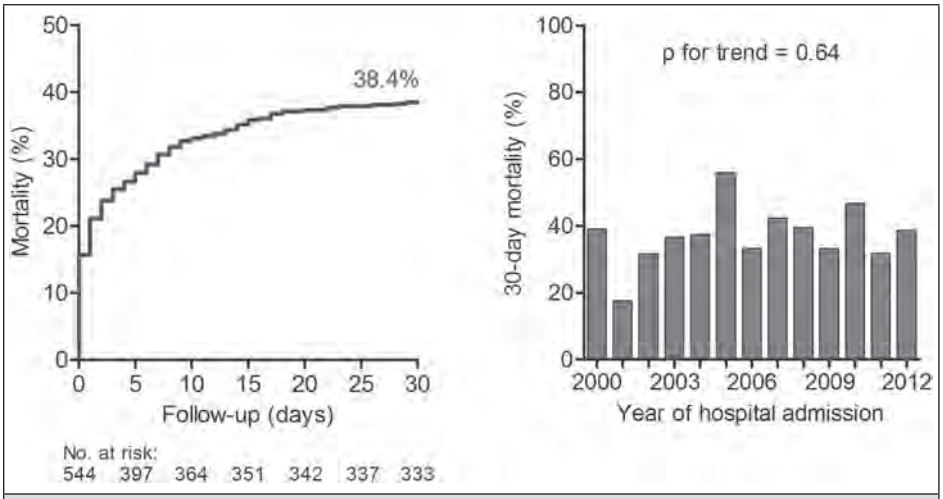

Figure 1. 30-day mortality
Left figure displays Kaplan-Meier curve of 30-day mortality. Right figure displays 30-day mortality over the years.

Table 2. Associations with 30-day mortality in univariable analysis

	OR (95%CI)	P
Age per year	1.05 (1.03-1.06)	<0.001
Men	0.53 (0.36-0.78)	0.001
History of diabetes	1.24 (0.78-1.99)	0.37
History of hypertension	0.79 (0.54-1.15)	0.22
History of prior MI	1.37 (0.89-2.09)	0.15
History of prior PCI	0.73 (0.39-1.36)	0.33
History of prior CABG	1.64 (0.75-3.61)	0.22
History of renal failure	1.78 (0.80-3.99)	0.16
Out-of-hospital cardiac arrest	1.49 (1.02-2.20)	0.041
Systolic blood pressure per 10 mmHg	0.95 (0.89-1.01)	0.097
Diastolic blood pressure per 10 mmHg	0.86 (0.78-0.95)	0.004
Mean arterial pressure per 10 mmHg	0.89 (0.81-0.97)	0.011
Heart rate per 10 bpm	1.04 (0.98-1.11)	0.21
Acute MI located anterior/lateral	1.14 (0.79-1.64)	0.50
Initial serum troponin T per 10 µg/L	1.13 (0.82-1.54)	0.45
Initial serum lactate per mmol/L	1.14 (1.09-1.21)	<0.001
Initial serum creatinine >ULN[a]	3.53 (2.39-5.20)	<0.001

[a] ULN for serum creatinine in men was 115 µmol/L, ULN for women was 90 µmol/L.
CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention; ULN, upper limit of normal.

38.4% (n=208) and did not change over the years (Figure 1). In univariable analysis, age, gender, out-of-hospital cardiac arrest, systolic blood pressure, diastolic blood pressure, mean arterial pressure, as well as initial serum lactate and serum creatinine level were associated with 30-day mortality (Table 2). The serum creatinine level had a non-linear association with 30-day mortality (increased risk was only observed in patients with creatinine levels above the ULN), and was therefore categorized using the ULN (i.e. 115 µmol/L for men and 90 µmol/L for women) as cut-off value (Supplemental Figure 1). In multivariable analysis where all variables in Table 2 were entered in a forward stepwise manner, only three parameters remained independent predictors of 30-day mortality: age, initial serum lactate level and initial serum creatinine level (Table 3).

Risk chart for initial risk assessment

The variables age, initial serum lactate level and initial serum creatinine level were used to develop the risk chart. Age was categorized into 4 groups (that were approximately similar in size) by using the following cut-off values: 55, 65 and 75 years (Supplemental Figure 1). Serum lactate was categorized into 4 groups by using the following cut-off values: 1.7 (1x ULN), 5.1 (3x ULN) and 8.5 (5x ULN) mmol/L. The OR per increasing cat-

Table 3. Independent predictors of 30-day mortality

	Continuous[a] OR (95%CI)	Categorized[b] OR (95%CI)	P
Initial serum lactate	1.17 (1.11-1.24)	1.93 (1.56-2.38)	<0.001
Age	1.05 (1.04-1.07)	1.67 (1.38-2.02)	<0.001
Initial serum creatinine >ULN		2.89 (1.90-4.37)	<0.001

The independent predictors were obtained by entering all baseline variables into a logistic regression model in a forward stepwise manner.

[a] Odds ratio per year increase of age and per mmol/L increase in lactate.

[b] Odds ratio per increasing category. Age was categorized into <55, 55-65, 65-75 and >75 years. Lactate was categorized into <1.7, 1.7-5.1, 5.1-8.5 and >8.5 mmol/L, representing <1x ULN, 1-3x ULN, 3-5x ULN and >5x ULN. ULN of creatinine is 115 µmol/L for men and 90 µmol/L for women.

ULN, upper limit of normal

egory of serum lactate level was 1.93 (95% CI 1.56-2.38), the OR per increasing category of age was 1.67 (95% 1.38-2.02) and the OR for serum creatinine >ULN was 2.89 (95% CI 1.90-4.37) (Table 3). The predicted risks of each possible combination of these three variables are displayed in the risk chart, and the risk is divided into 5 categories ranging from 0-20% to 80-100% risk of 30-day mortality (Figure 2 and supplemental Figure 2). The calibration plot of the risk chart showed a close relationship between expected and observed mortality (Figure 3). The c-index of the risk chart was 0.75 (Figure 4). The discriminatory power was similar in the unimputed dataset in which patients with missing

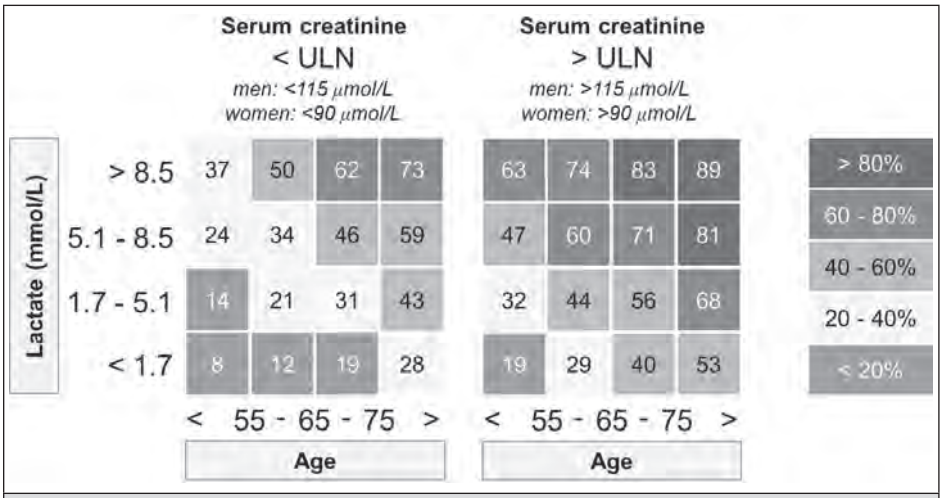

Figure 2. Risk chart for initial risk assessment of 30-day mortality in cardiogenic shock from ST-elevation myocardial infarction

ULN, upper limit of normal.

See also Appendix: Color figure 6.

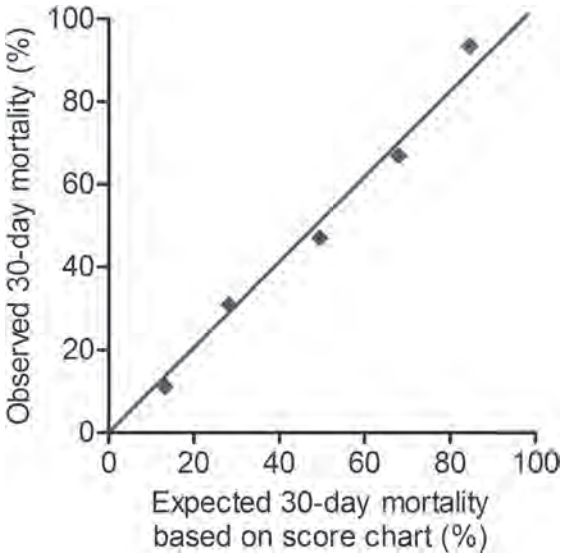

Figure 3. Calibration plot
Calibration plot of actual incidence versus predicted probability of 30-day mortality in each risk category of the risk chart. The diagonal line indicates perfect calibration.

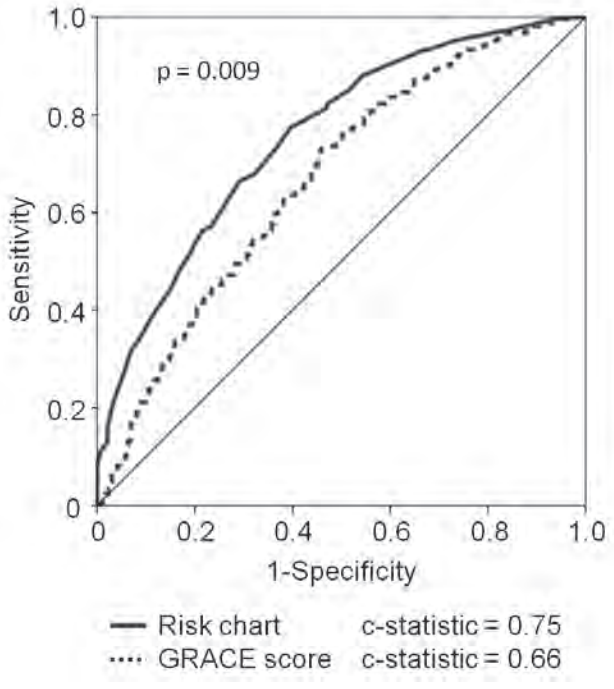

Figure 4. Discriminatory power on receiver operation characteristic curve
GRACE, Global Registry of Acute Coronary Events.

variables were excluded (c-index 0.74, p=0.69). The GRACE score achieved a c-index of 0.66 in our patients population, which was significantly lower than that of our risk chart (p=0.009; Figure 4).

Variables obtained from coronary angiography and clinical course (data that were not yet available at time of hospital admission) that were predictive for 30-day mortality independent of age, initial serum lactate and serum creatinine levels were: a lesion in the left main coronary artery, need for mechanical ventilation and occurrence of ventricular fibrillation or tachycardia (Supplemental Table 1). However, adding these variables to the risk chart model did not significantly improve discriminatory power (c-index 0.77, p=0.48).

DISCUSSION

This study demonstrates that 30-day mortality in patients with cardiogenic shock from STEMI undergoing primary PCI can be accurately predicted at the time of hospital admission by using a simple risk scoring chart that includes only three clinical variables that may be readily available at the time of initial assessment (age, initial serum lactate and creatinine levels). The risk chart has good predictive accuracy for 30-day mortality in patients with cardiogenic shock from STEMI who are selected for primary PCI, and performed better than an established risk score that is used for ACS patients in general (i.e. GRACE score). Adding further information obtained from coronary angiography or during hospital stay did not significantly improve the predictive accuracy of the risk chart.

The current study shows that 30-day mortality of patients with cardiogenic shock from STEMI who undergo primary PCI did not change in the last decade. Until now, early revascularization (e.g. primary PCI) remains the only therapy that is proven to reduce in-hospital mortality.[1,5,14] Clinical trials have failed to demonstrate a survival benefit of mechanical circulatory assist devices, including IABP and percutaneous LVADs, when routinely used in STEMI patients with cardiogenic shock.[8,15] However, the use of circulatory assist devices is believed to be potentially effective in selected patients, especially in patients in an early stage of cardiogenic shock at which time the organ dysfunction is considered to be still reversible.[3] Therefore, early risk assessment might be important in these patients to select patients that might benefit from advanced mechanical circulatory assist devices such as ECMO or percutaneous LVAD.

The Should We Emergently Revascularize Occluded Coronaries For Cardiogenic Shock (SHOCK) study has previously developed a prediction model for 30-day mortality in patients with cardiogenic shock from STEMI. This model included 8 variables: age, anoxic brain damage, end-organ hypoperfusion, shock on admission, prior CABG, non-inferior

MI, creatinine ≥1.9 mg/dL and systolic blood pressure.[16] Serum lactate, which was a strong and independent predictor in our risk chart, was not available as parameter in the SHOCK study. The c-index of the SHOCK prediction model was 0.74, which is comparable to that of our risk chart. However, a number of the 8 variables that are used in the SHOCK risk model are less objective (anoxic brain damage, end-organ hypoperfusion) than the variables used in our risk chart. Moreover, these variables cannot be assessed at the time of hospital admission. Furthermore, the patients in the SHOCK study were included between 1993 and 1999, and the treatment of these patients (i.e. thrombolysis) may not be representative for current clinical practice. Therefore, our risk chart may be more applicable and more easy to use for initial risk assessment in contemporary clinical practice.

Some limitations of our study need to be acknowledged. Firstly, a few variables that are of particular interest in patients with cardiogenic shock (e.g. serum lactate) were retrospectively collected from patient records, since such information was not routinely registered in the prospective registry of STEMI patients. Missing data was more common in the retrospectively collected variables. However, multiple imputation has been shown to produce unbiased parameter estimates which reflect the uncertainty associated with estimating missing data, and has also been used by previous studies to develop risk scores, including the GRACE score.[4] Secondly, our risk chart has not been validated in an independent dataset. Although the study population is representative for cardiogenic shock patients in current clinical practice in tertiary hospitals, external validation of the risk chart is warranted. Caution is urged in extrapolating the results to cardiogenic shock patients who did not have STEMI and who were not selected for primary PCI. Clinical trials may be less suitable for validation of the risk chart, because (strict) in- and exclusion criteria may lead to a selected study population that does not reflect the overall patient population in daily clinical practice.[17] Finally, additional (invasive) measurements of hemodynamic parameters, microcirculation and organ dysfunction may further improve outcome prediction. In clinical practice, the patient's estimated mortality risk may change when such information becomes available (in a later stage during hospital stay). Again, the current prediction model was not developed to be a comprehensive model with optimal predictive ability, but rather to be an easy-to-use risk chart with a fair predictive ability that can be used for initial risk assessment by physicians that are confronted with this dreadful complication of STEMI in every-day clinical practice. Further research is warranted to investigate which patient risk category may have the largest benefit from mechanical circulatory support devices. It may be speculated that patients with a "medium" risk of 30-day mortality may have more benefit from mechanical circulatory support devices than low risk patients (who may have a good clinical outcome without any additional hemodynamic support) and high risk patients (who may have irreversible cardiogenic shock).

In conclusion, 30-day mortality of patients with cardiogenic shock from STEMI can be well predicted in an early stage at hospital admission using a risk chart with only three clinical parameters that are directly available at the time of the initial assessment. These three variables were age, initial serum lactate level and initial serum creatinine level, and they were strong and independent predictors of 30-day mortality in this patient population. A simple risk chart that includes these three variables was able to stratify patient's mortality risk into categories ranging from 0-20% to 80-100%, and had better performance than the GRACE risk score that is used for ACS patients in general. The risk chart may be a useful tool in clinical practice for initial risk assessment in patients with cardiogenic shock from STEMI. Furthermore, the risk chart may also be used in future clinical trials (e.g. to test new circulatory assist devices) to select eligible cardiogenic shock patients of a particular risk category.

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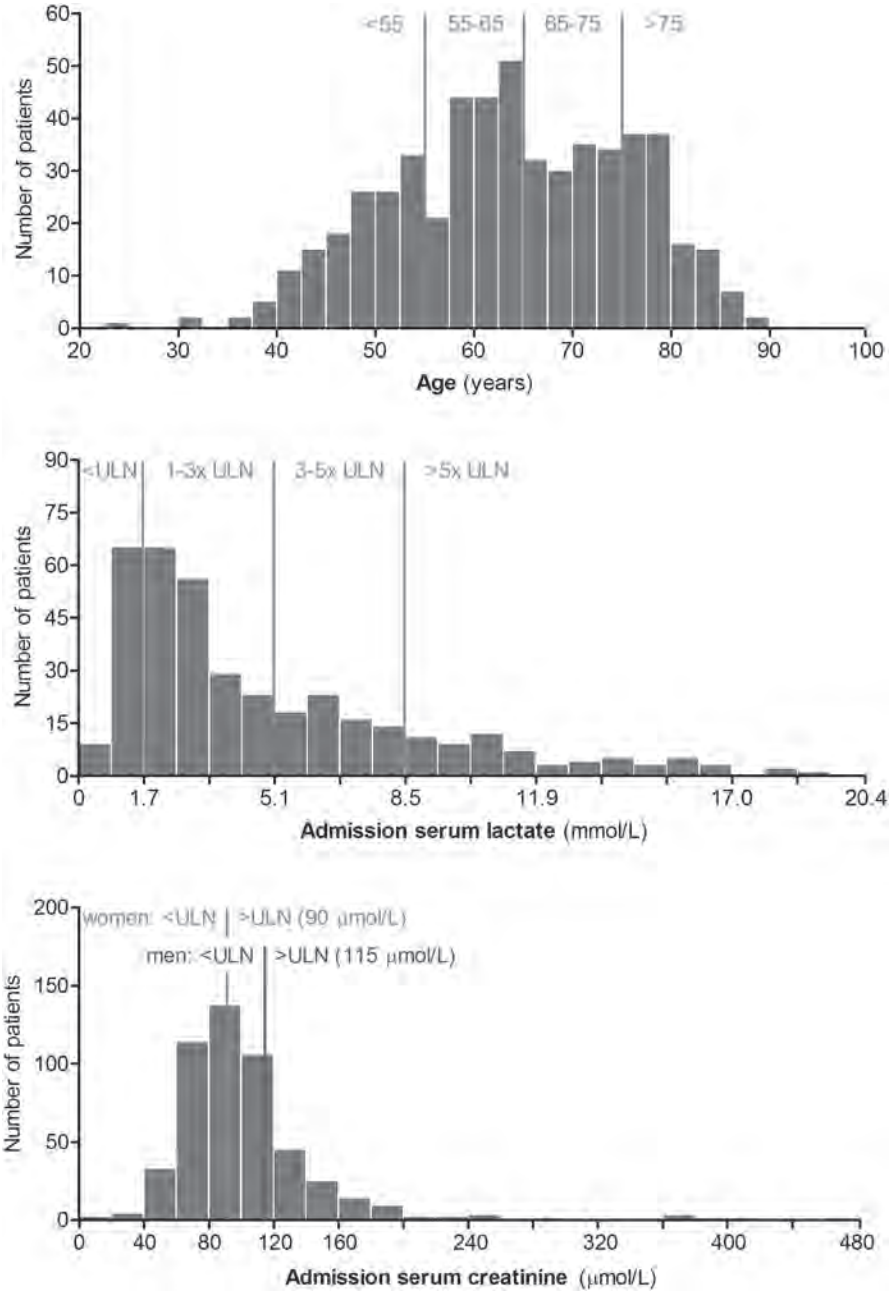
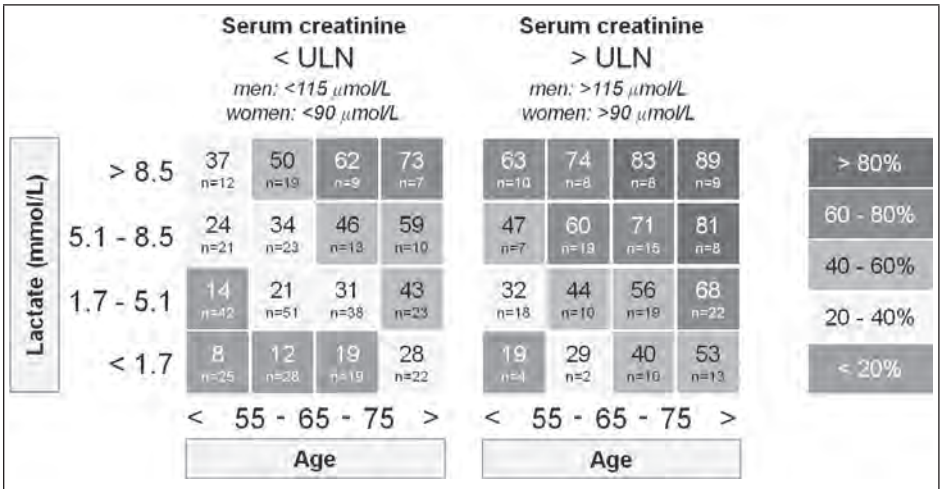
SUPPLEMENTAL FIGURES AND TABLES



Supplemental Figure 1. Cut-off values for categorization of age and serum lactate showed in histogram
ULN, upper limit of normal.



Supplemental Figure 2. Risk chart for initial risk assessment of 30-day mortality with sample size of each cell.

Supplemental table 1. Additional predictors obtained from coronary angiography and during stay at the intensive cardiac care unit

	OR (95%CI)[a]	P
Coronary angiography and primary PCI		
Lesion in right coronary artery	0.89 (0.57-1.38)	0.59
Lesion in left anterior descending	0.72 (0.49-1.07)	0.11
Lesion in left circumflex	1.22 (0.76-1.96)	0.40
Lesion in left main	2.48 (1.36-4.52)	0.003
Lesion in bypass graft	0.89 (0.25-3.17)	0.86
Multivessel disease	1.52 (0.98-2.37)	0.062
Primary PCI performed	0.85 (0.29-2.49)	0.77
Multivessel treatment	1.16 (0.68-1.98)	0.58
Clinical course during intensive cardiac care unit stay		
IABP support	0.83 (0.51-1.36)	0.46
Need for mechanical ventilation	2.53 (1.67-3.86)	<0.001
Ventricular fibrillation or tachycardia	1.64 (1.06-2.55)	0.026
Peak TnT per 10 µg/L	1.10 (0.94-1.27)	0.23
Peak CK per 1000 U/L	1.03 (0.98-1.08)	0.30

[a] Adjusted for age, initial serum lactate and initial serum creatinine.

Chapter 17

Impaired microcirculation predicts poor outcome of patients with acute myocardial infarction complicated by cardiogenic shock

Den Uil CA, Lagrand WK, Van Der Ent M, Jewbali LSD, Cheng JM, Spronk PE, Simoons ML.

Eur Heart J. 2010;31(24):3032-3039.

ABSTRACT

Aims: We investigated the relationship between sublingual perfused capillary density (PCD) as a measure of tissue perfusion and outcome (ie, occurrence of organ failure and mortality) in patients with cardiogenic shock from acute myocardial infarction.

Methods and results: We performed a prospective study in 68 patients. Using Side-stream Dark Field imaging, PCD was measured after hospital admission (T0, baseline) and 24 hours later (T1). We compared patients with baseline PCD ≤ median to patients with baseline PCD > median. Sequential Organ Failure Assesment (SOFA) scores were calculated at both time points. Kaplan-Meier 30-day survival analyses were performed and predictors of 30-day mortality were identified. Baseline PCD was a predictor of the change in SOFA score between T0 and T1 (ΔSOFA; ρ=-0.25, p=0.04). Organ failure recovered more frequently in patients with PCD > median (>10.3 $mm.mm^{-2}$; n=33) than in patients with PCD ≤ median (n=35) (52% vs. 29%, p<0.05). Twenty-two patients (32%) died: 17 patients (49%) with PCD ≤ median vs. 5 patients (15%) with PCD > median (p=0.004). After adjustment, cardiac power index (OR 0.48, 95%CI [0.24-0.94]), and PCD (OR 0.65, 95%CI [0.45-0.92]) remained significant predictors of 30-day outcome. Patients with baseline sublingual PCD ≤ median that improved at T1, had a considerable better prognosis relative to patients who had a persistently low PCD.

Conclusion: Diminished sublingual PCD, at baseline or following treatment, is associated with development of multi-organ failure, and is a predictor of poor outcome in patients with acute myocardial infarction complicated by cardiogenic shock.

INTRODUCTION

Cardiogenic shock is the most important cause of death in patients hospitalized with acute myocardial infarction.(1) Although in-hospital survival of cardiogenic shock is improving with more intensive treatment, 30-day mortality rate remains high.(2, 3) Because cardiogenic shock is caused by extensive myocardial infarction and a decrease of cardiac output, timely reperfusion and normalization of hemodynamic parameters are the main objectives in the treatment of cardiogenic shock.(4) However, it has been shown that 45% of patients dying from cardiogenic shock have a preserved cardiac index, indicating that optimization of macro-hemodynamic parameters alone may fail to save the patient. (5, 6) In line with these data, post-hoc analysis of data from the SHOCK-trial demonstrated that the classic notion of systemic vasoconstriction as a response to low arterial pressure did not apply to all patients with cardiogenic shock. Indeed a large variability in cardiac index and systemic vascular resistance (SVR) has been reported among patients with cardiogenic shock , even despite application of vasopressor therapy.(7) These data indicate that cardiogenic shock is a primarily cardiac problem leading to subsequent derangements in the entire circulatory system.(8) It is currently accepted that cardiogenic shock causes a systemic inflammatory response (SIRS), which is characterized by the release of inflammatory mediators and neurohormones as well as by alterations in tissue microvasculature, which may result in multi-organ failure.(9, 10) Indeed, several studies have reported that markers of SIRS are predictive of short-term mortality in cardiogenic shock.(11-14) Nevertheless, the mechanisms involved in the pathogenesis of multiple organ failure in cardiogenic shock patients remain largely unknown. Possibly impaired splanchnic perfusion at the microvascular level, modulated by the severity of heart failure, by the degree of SIRS, and by the administration of vasoactive agents, plays an important role in the pathogenesis of multi-organ failure and the persistence of shock. (15, 16)

Sublingual microcirculation is a surrogate marker of splanchnic perfusion and can be measured at the bedside using novel imaging technology.(16-18) Therefore we investigated the relationship between sublingual microcirculation and outcome (i.e., (change in) SOFA score, occurrence of multi-organ failure and mortality) in patients with acute myocardial infarction complicated by cardiogenic shock.

METHODS

Study design

This prospective study was conducted at the Intensive Cardiac Care Unit (ICCU) of the Thoraxcenter, Erasmus University Medical Center, Rotterdam, the Netherlands. We in-

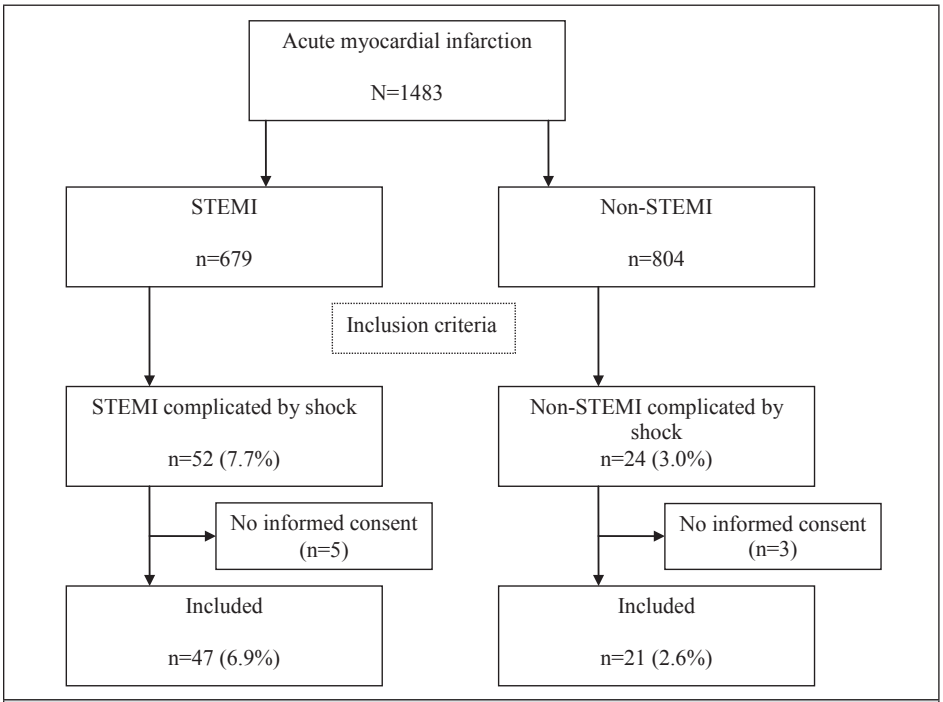


Figure 1. Study flow chart.
Abbreviation: STEMI, ST-segment elevation myocardial infarction

cluded patients who were admitted with acute myocardial infarction complicated by cardiogenic shock in the time period November 2007-April 2009 (Flow chart: Figure 1). Cardiogenic shock was defined as sustained hypotension (systolic blood pressure <90 mm Hg) induced by heart failure together with clinical signs of hypoperfusion (i.e., cold extremities, oliguria or altered mental state), not responsive to fluid resuscitation.(4) The institutional ethical committee approved the protocol, and written informed consent was obtained from each patient or, in case of patients who were sedated, from a relative authorized to consent on behalf of such a patient.

Hemodynamic monitoring

All patients were monitored with a radial artery catheter (arterial cannula with FloSwitch, Ohmeda, Swindon, UK). Forty-eight (71%) patients were monitored with a pulmonary artery catheter (PAC: Becton Dickinson Criticath SP5107H, Sandy, UT, USA or CCOmbo, Edwards Lifesciences, Saint-Prex, Switzerland). In the remaining patients, central venous pressure was measured via a three-lumen central venous catheter (Multicath; Laboratoires Pharmaceutiques Vygon, Ecouen, France), inserted into the right internal jugular vein. In these patients, cardiac index (CI) was calculated according to the Cuschieri for-

mula, which shows close correlation with the cardiac index measured with a pulmonary artery catheter.(19)

Data collection

Data collection included central body temperature, heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP), mean pulmonary artery pressure (MPAP), cardiac index (CI), systemic vascular resistance (SVR), lactate level and mixed-venous oxygen saturation (SvO_2). When no pulmonary artery catheter was available, we estimated mixed-venous oxygen saturation by measuring venous oxygen saturation from blood sampled from the central venous line. Systemic vascular resistance (SVR) was calculated as (MAP-CVP)*80/cardiac output. Cardiac power index (CPI) was computed as mean arterial pressure*CI/451. Glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) equation.

Microcirculatory assessment and analysis

The Sidestream Dark Field (SDF) imaging device (MicroScan; Microvision Medical, Amsterdam, The Netherlands) was used to obtain 2-dimensional video images of sublingual microcirculatory blood flow as described previously.(20) In short, the camera emits green light that is absorbed by red blood cells within microvessels. In this way, red blood cells are used as the contrast agent to visualize sublingual blood flow in patent microvessels. Per time point, 3-5 steady video sequences of at least 20 seconds duration were obtained, stored and analyzed in a randomized and blinded fashion. Quantification of the images was done using dedicated software (Automated Vascular Analysis 3.0, MicrovisionMedical, Amsterdam, the Netherlands) by a blinded investigator. Perfused capillary density (PCD) was calculated by measuring total length of perfused capillaries divided by image area. Capillaries were regarded as perfused if they had either of the following flow classifications obtained by visual inspection: sluggish, continuous or hyperdynamic.(21, 22) Unperfused capillaries (ie, capillaries with absent or intermittent perfusion) were judged not to take part of the circulation and were not taken into account. Since SDF imaging enables visualization of flowing intravascular erythrocytes rather than microvessel walls, an increase in PCD was regarded as capillary recruitment. This approach has been validated previously and within-patient variability and inter- and intra-observer variability's of the technique are low.(23-25) Capillaries were defined as microvessels with a diameter less than 20 µm. Reference values for sublingual PCD in control patients (ie, patients awaiting cardiac surgery who were not in shock) have been reported previously, i.e. ≥ 11.7 $mm.mm^{-2}$ (2.5 percentile) (26, 27)

Image acquisition is particularly comfortable in patients who are sedated and intubated, whereas in patients who are awake movement of the tongue may more easily result in movement artefacts. However, we and other investigators extensively reported the

feasibility of using this device in critically ill patients in several reports albeit in research settings.(25, 28-30) In addition, Arnold et al. (30) recently compared a real-time point-of-care (POC) determination of the microcirculation to conventional off-line analysis. The POC assessment of microcirculation was 94% sensitive and 92% specific for detecting impaired microvascular flow.

Study protocol

The sublingual microcirculation was investigated as soon as possible after the patient's admission to the ICCU and after informed consent had been obtained (T0, baseline). Measurements were repeated 24 hours after the first measurement or earlier, pending the individual clinical course of the patient (i.e., significant deterioration which might lead to death within 24 hours). In addition, at both time points, all components of the Sequential Organ Failure Assesment (SOFA) score were calculated, with the exception of the central nervous system parameters, because the majority of the patients received central nervous system depressant drugs at the time of evaluation.(14, 31) The total SOFA score was calculated by summing the scores for each of the components (i.e. cardiac, renal, respiratory, coagulation, and liver).(32)

Follow-up

Vital status at 30 days was registered for all patients. In patients who were transported to other hospitals or were discharged during the 30 days following baseline measurements, vital status was acquired from municipal civil registries. Response rate was 100% and no patients were lost during 30 days of follow-up.

Statistical analysis

Statistical analyses were performed using SPSS 15.0 for Windows. Categorical variables are presented as absolute numbers with percentages. Continuous variables are presented as mean ± standard deviation. Non-normally distributed continuous variables are presented as median [interquartile range]. Because this study is the first study that presents PCD measurements in patients with cardiogenic shock, we decided a priori to compare the patients with baseline sublingual PCD ≤ median with the patients in whom baseline sublingual PCD was > median. Categorical variables were compared by chi-square test or Fisher's exact test, when appropriate. Differences between groups were tested with Student's T-test or the Mann-Whitney test, when appropriate. Changes between time points were tested with the paired T-test or the Cochran's Q-test, when appropriate. Correlations between variables were investigated with Pearson or Spearman correlation test, when appropriate. Kaplan-Meier cumulative 30-day survival was calculated and Kaplan-Meier survival curves were compared by the Log-rank test. Univariate and multivariate logistic regression analyses were performed to identify predictors of

30-day all-cause mortality. Final results are presented as unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (95%CI). The multivariate logistic regression model selection was done with backward stepwise method starting with the following variables: Age >75 years, cardiac power index, baseline SOFA score, nitroglycerin, left main coronary artery occlusion, left ventricular ejection fraction <30%, significant mitral valve regurgitation, and sublingual PCD. Variables that remained significantly associated with 30-day mortality were part of the regression equation and are presented. The multivariate model was confirmed by using forward stepwise selection. We selected the variables based on differences in baseline characteristics among both subgroups and on previous reports on prognosticating factors in cardiogenic shock.(25, 26, 33-35) Given our hypothesis, we further added sublingual PCD and baseline SOFA score, which consists of multiple variables itself, including inotropic and vasopressor support. Sublingual PCD was entered into the model as a continuous variable. Cardiac power index was categorized into units of 0.10 $W.m^{-2}$.(33) All tests were two-sided. A p-value <0.05 was regarded statistically significant.

RESULTS

We investigated 68 patients with acute myocardial infarction complicated by cardiogenic shock; 47 patients had a STEMI and 21 patients had a non-STEMI (Figure 1, Table 1). Mean age was 60 ± 14 years and 69% of the patients were male. Ninety-seven percent of the patients still met the inclusion criteria during baseline measurements. The remaining patients (n=2) received high dosages of vasopressors, which resulted in systolic blood pressures >90 mm Hg. Median PCD was 10.3 $mm.mm^{-2}$ (range: 4.3-15.9 $mm.mm^{-2}$; please note the Supplementary material online for video samples of high and low sublingual PCD). Patients with PCD ≤ median (n=35) were less frequently older than 75 years as compared to patients with sublingual PCD > median (n=33; 9% vs. 30%, p=0.03, Table 1). Patients with PCD ≤ median more frequently had an ejection fraction below 30% (74% vs. 42%, p=0.01). Median baseline SOFA score was not significantly different between both groups. Patients with a PCD ≤ median had a higher PCWP (23 [18-25] mmHg vs. 18 [14; 22] mmHg, p=0.04) than patients with a PCD > median (Table 2).

Baseline sublingual perfused capillary density

Baseline PCD correlated to MAP (ρ=0.34, p=0.004), PCWP (ρ=-0.32, p=0.03), and to CPI (ρ=0.25, p=0.04) but not significantly to baseline SOFA score or to other parameters listed in Table 2. Baseline PCD predicted the change in SOFA score between T0 and T1 (ΔSOFA; ρ=-0.25, p=0.04). Patients with baseline sublingual PCD > median improved more frequently in total SOFA score (52% vs. 29%, p<0.05) and in cardiac SOFA score

Table 1. Baseline characteristics.

	All patients (n=68)	PCD ≤median¶ (n=35)	PCD >median¶ (n=33)	P-value
Age, yrs (mean ± SD)	60 ± 14	59 ± 12	62 ± 15	0.24
Age >75 years	13 (19%)	3 (9%)	10 (30%)	0.03
Gender, male	47 (69%)	24 (69%)	23 (70%)	0.99
Proportion of patients still meeting inclusion criteria during baseline measurements*	66 (97%)	34 (97%)	32 (97%)	0.99
CV risk factors				
Hypertension	27 (40%)	11 (31%)	16 (49%)	0.22
Diabetes mellitus	21 (31%)	12 (34%)	9 (27%)	0.61
Current smoking	16 (24%)	10 (29%)	6 (18%)	0.40
Dyslipidemia	18 (27%)	9 (26%)	9 (27%)	0.99
Electrocardiography				
Non-STEMI	21 (31%)	11 (31%)	10 (30%)	0.99
STEMI	47 (68%)	24 (69%)	23 (70%)	
Laboratory				
Hemoglobin, mmol/L	6.6 [5.9-7.7]	6.6 [5.8-7.7]	6.6 [6.0-7.7]	0.78
WBC count, x10^9/L (median, IQR)	11.9 [9.8-17.3]	12.9 [9.8-18.0]	11.4 [8.8-17.1]	0.35
CRP, mg/L (median, IQR)	55 [15-138]	55 [18-136]	49 [9-149]	0.81
GFR (ml/min)	58 [37-83]	55 [39-77]	66 [33-90]	0.55
NT-proBNP, pg/mL (median, IQR)	3775 [1316-9140]	4127 [1958-10,873]	2839 [1186-8653]	0.54
Peak Creatine kinase, U/L (median, IQR)	3455 [403-6786]	3891 [355-7221]	3093 [413-5948]	0.47
Peak Troponin T, ug/L (median, IQR)	5.7 [1.2-12.9]	7.8 [1.8-13.4]	4.0 [0.6-13.8]	0.54
Echocardiography				
Ejection fraction <30%	40 (59%)	26 (74%)	14 (42%)	0.01
Moderate-severe MR	17 (25%)	10 (29%)	7 (21%)	0.58
Angiography				
No angiography	6 (9%)	4 (11%)	2 (6%)	0.67
1-vessel disease	17 (25%)	7 (20%)	10 (30%)	
2-vessel disease	15 (22%)	7 (20%)	8 (24%)	
3-vessel or LM disease	30 (44%)	17 (49%)	13 (39%)	
Occlusion of LM	15 (24%)	8 (26%)	7 (23%)	0.99
Treatment				
ASA	67 (99%)	35 (100%)	32 (97%)	0.49
Clopidogrel	52 (77%)	28 (80%)	24 (73%)	0.57
UFH/LMWH	68 (100%)	35 (100%)	33 (100%)	0.99
GP IIb/IIIa inhibitors	10 (15%)	5 (14%)	5 (15%)	0.99
Enoximone and/or dobutamine and/ or dopamine ≤5#	27 (40%)	16 (46%)	11 (33%)	0.31
Dopa >5 or Norepi ≤0.1#	16 (24%)	6 (17%)	10 (30%)	
Dopa >15 or Norepi >0.1#	15 (22%)	8 (23%)	7 (21%)	
Nitroglycerin	9 (13%)	7 (20%)	2 (6%)	0.15
Mechanical ventilation	49 (72%)	22 (63%)	27 (82%)	0.11
IABP	30 (44%)	18 (51%)	12 (36%)	0.23
ECMO	3 (4%)	1 (3%)	2 (6%)	0.61
Revascularization				
No revascularization	14 (21%)	6 (17%)	8 (24%)	0.74
Thrombolysis	0 (0%)	0 (0%)	0 (0%)	
PCI	49 (72%)	26 (74%)	23 (70%)	
CABG	5 (7%)	3 (9%)	2 (6%)	
TIMI flow after PCI	3 [3-3]	3 [3-3]	3 [3-3]	0.22

Table 1. **Baseline characteristics** (continued)				
	All patients (n=68)	**PCD ≤median¶ (n=35)**	**PCD >median¶ (n=33)**	**P-value**
SOFA score (median, IQR)				
Total	5 [4-7]	5 [3-8]	6 [4-7]	0.96
Cardiac subscore	2 [2-3]	2 [2-3]	3 [2-3]	0.65
Renal subscore	1 [0-1]	1 [0-1]	0 [0-2]	0.70
Respiratory subscore	1 [1-2]	2 [1-2]	1 [1-2]	0.59
Coagulation subscore	0 [0-1]	0 [0-1]	0 [0-1]	0.86
Liver subscore	0 [0-0]	0 [0-0]	0 [0-0]	0.62
Timing of baseline measurements				
Time from AMI, hours	16 [6-20]	16 [9-20]	12 [4-22]	0.36
Time from shock onset, hours	5 [3-10]	5 [4-8]	4 [2-11]	0.39

Abbreviations: SD, standard deviation; NS, non-significant; AMI, acute myocardial infarction; CV, cardiovascular; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; NT-proBNP, N-terminal proB-type natriuretic peptide; IQR, interquartile range; WBC, white blood cell; CRP, C-reactive protein; GFR, glomerular filtration rate; MR, mitral valve regurgitation; LM, left main coronary artery; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; SOFA, sequential organ failure assessment. *systolic blood pressure < 90 mm Hg and clinical signs of hypoperfusion; #dosages in ug/kg/min; ¶ median PCD = 10.3 $mm.mm^{-2}$.

(61% vs. 34%, p=0.03) at 24 hours, as compared to patients with sublingual impaired PCD ≤ median.

Twenty-two patients (32%) died during 30 days of follow-up. All these patients died in the hospital. Of the patients who had a PCD ≤ median, 17 (49%) died vs. 5 (15%) of the patients with PCD > median (p=0.004, Figure 2). Inverse sublingual PCD as a continuous

Table 2. **Baseline hemodynamic parameters.**				
	All patients (n=68)	**PCD ≤median# (n=35)**	**PCD >median# (n=33)**	**P-value**
HR, bpm	93 [72-104]	92 [71-106]	93 [72-104]	0.80
MAP, mmHg	69 [61-70]	66 [58-70]	70 [64-70]	0.07
CVP, mmHg	15 [12-18]	16 [12-19]	14 [13-16]	0.23
PCWP, mmHg*	21 [16-24]	23 [18-25]	18 [14-22]	0.04
MPAP, mmHg*	28 [24-34]	30 [24-37]	27 [24-30]	0.18
CI, $L.min^{-1}.m^{-2}$	2.5 [2.1-2.9]	2.4 [1.8-2.9]	2.7 [2.1-2.9]	0.44
CPI, $W.m^{-2}$	0.35 [0.26-0.42]	0.33 [0.24-0.39]	0.38 [0.30-0.42]	0.11
SVR, $dynes.sec.cm^{-5}$	1075 [825-1242]	1075 [798-1237]	1052 [850-1256]	0.79
SvO_2, %	66 [61-73]	65 [60-70]	68 [62-75]	0.12
Lactate, $mmol.L^{-1}$	2.8 [2.0-4.3]	2.9 [1.8-4.5]	2.8 [2.2-4.8]	0.58

Abbreviations: HR, heart rate; NS, non-significant; MAP, mean arterial pressure; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; MPAP, mean pulmonary artery pressure; CI, cardiac index; CPI, cardiac power index; SVR, systemic vascular resistance; SvO2, central-venous oxygen saturation. * Data available in 48 (71%) of the patients; # median PCD = 10.3 $mm.mm^{-2}$.

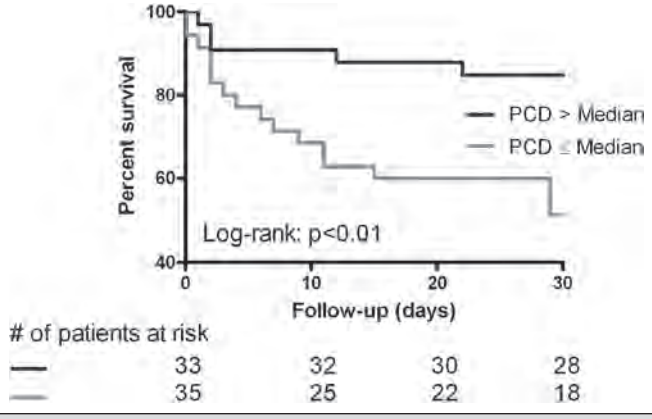


Figure 2. Kaplan-Meier survival curve stratified according to perfused capillary density at baseline. Median PCD = 10.3 mm.mm^{-2}.

parameter had a greater predictive value on 30-day mortality than baseline SOFA score (area under the receiver operator characteristic curve 0.75 vs. 0.56). The threshold best predicting 30-day mortality was 10.0 mm.mm^{-2} (area under the curve of 0.72 vs. 0.69 when the median (10.3 mm.mm^{-2}) was used). Left ventricular ejection fraction < 30% (OR 3.40, 95%CI [1.07-10.8]) was significantly associated with 30-day mortality, whereas CPI (OR 0.42, 95%CI [0.23-0.78]) and sublingual PCD (OR 0.61, 95%CI [0.44-0.84]) were significantly associated with improved 30-day survival. After adjustment, CPI (OR 0.48, 95%CI [0.24-0.94]), and sublingual PCD (OR 0.65, 95%CI [0.45-0.92]) remained significant predictors of 30-day outcome (Figure 3). Survival within 30 days according to quartile of baseline sublingual PCD is shown in Figure 4.

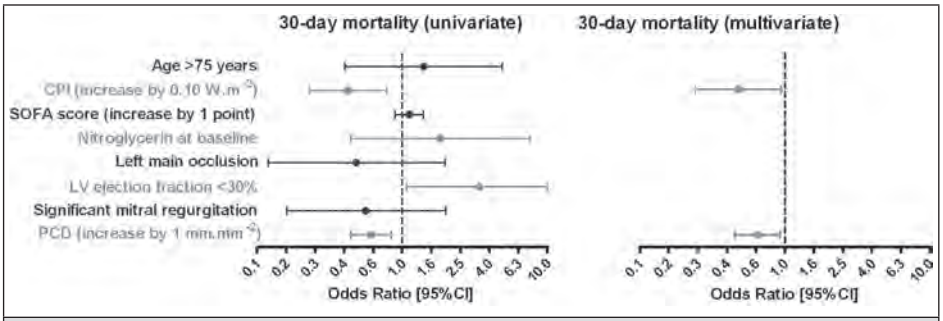


Figure 3. Predictors of 30-day mortality (univariate and multivariate analyses).
The multivariate logistic regression model selection was done with backward stepwise method starting with the following variables: Age >75 years, cardiac power index, baseline SOFA score, nitroglycerin, left main coronary artery occlusion, left ventricular ejection fraction <30%, significant mitral valve regurgitation, and sublingual PCD. Variables that remained significantly associated with 30-day mortality were part of the regression equation and are presented. The multivariate model was confirmed by using forward stepwise selection.

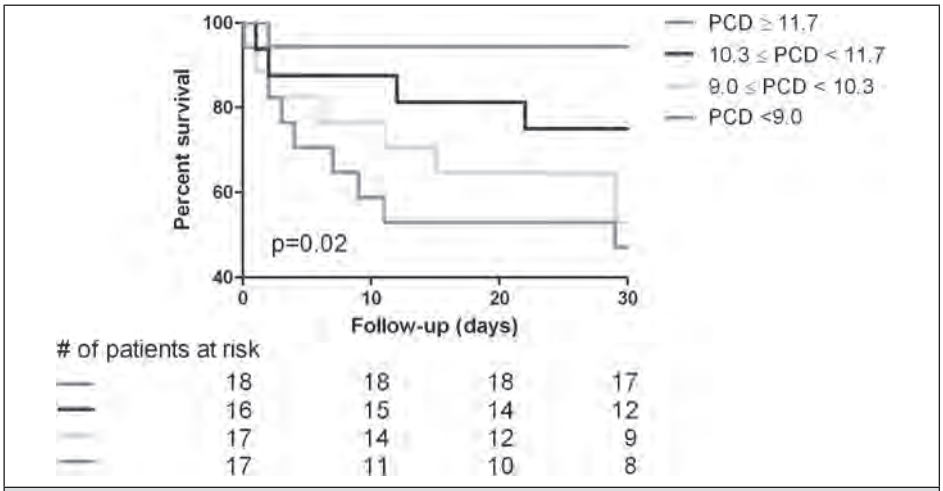


Figure 4. Kaplan-Meier survival curve stratified according to quartile of baseline sublingual perfused capillary density.

Association between changes in PCD and outcome

In 54 patients (79%), PCD measurements were repeated (T1). In the remaining patients (n=14), PCD measurements at T1 were not possible. One patient died immediately after the first measurements, 5 patients were sent back to the referring hospital before T1 and in 8 patients there was no investigator available to perform the measurements. Overall, sublingual PCD tended to increase at T1 relative to T0 (10.3 ± 2.2 mm.mm^{-2} at T0 vs. 10.9 ± 2.2 mm.mm^{-2} at T1, p=0.09). At time point T0, 27% of patients had a PCD ≥ 11.7 mm.mm^{-2} (reference value in control patients) and at T1, 43% of patients reached reference values (T0 vs. T1, p<0.05). Changes in sublingual PCD were inversely correlated to changes in CVP (ρ=-0.38, p=0.009). There was a modest correlation between PCD measured at 24 hours and SOFA scores at T1 (ρ=-0.40, p=0.003). In the total study group no significant correlation between changes in PCD and changes in SOFA score was found. However, patients who had a PCD ≤ median at both time points had higher SOFA scores at T1 relative to patients who had a sublingual PCD > median at T0 and T1 (7 [4-8] vs. 4 [3; 5], p=0.03). Survival of patients stratified to the level of PCD at both time points is shown in Figure 5. Patients who had a PCD ≤ median at baseline, which improved at T1 ("low-high"), had a significant better prognosis as compared to patients who had a persistently low PCD ("low-low"). When patients in whom no second measurement was performed were regarded as the sicker patients (i.e. PCD T1 ≤ median), results were identical. Finally, increase of PCD was significantly associated with a better outcome (OR 0.73, 95%CI [0.54-0.99]).

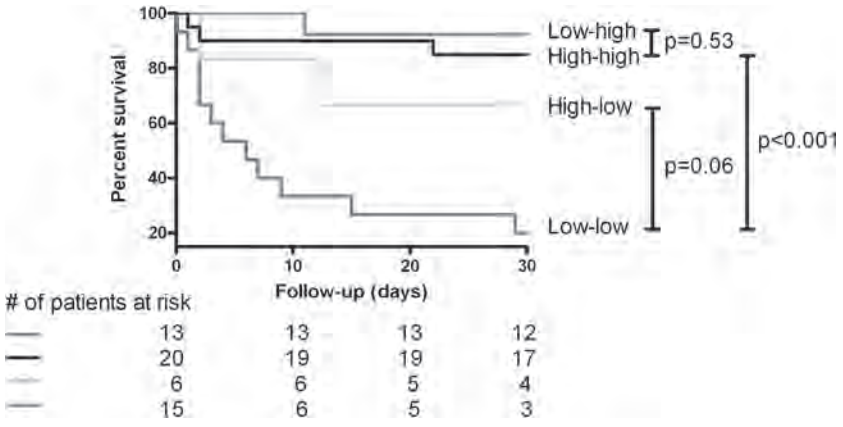


Figure 5. Kaplan-Meier survival curve of short-term survival of cardiogenic shock patients stratified according to sublingual perfused capillary density at baseline and after 24 hours.
Low-high: PCD ≤ median at T0, PCD > median at T1. High-high: PCD > median at T0, PCD > median at T1. High-low: PCD > median at T0, PCD ≤ median at T1. Low-low: PCD ≤ median at T0, PCD ≤ median at T1.

DISCUSSION

In this study, we demonstrated that patients with cardiogenic shock from acute myocardial infarction who had a sublingual PCD ≤ median had a higher risk to die. Baseline PCD was a significant predictor for change in SOFA-score within the next 24 hours. In addition, sublingual PCD at 24 hours correlated to SOFA score at T1. Patients with a higher baseline sublingual PCD were more likely to improve in total SOFA score as well as in cardiac SOFA subscore at 24 hours. Furthermore, baseline PCD was strongly and independently associated with 30-day outcome. Finally, in a large subgroup of patients in whom measurements were repeated, we demonstrated that patients who had a sublingual PCD ≤ median at baseline as well as after 24 hours were at high risk of poor outcome, as opposed to those patients in whom microcirculation recovered within 24 hours. In the latter patients, survival rates were similar to these of patients with PCD > median at both time points.

Using a semi-quantitative analysis technique, De Backer et al. previously described sublingual microcirculatory alterations in 31 patients with cardiogenic shock.(36) The authors reported a weak correlation of the proportion of perfused capillaries with mean arterial pressure, which is in line with our findings. We also found a weak correlation between sublingual PCD and cardiac power index. Such relative dissociation between macrocirculation (hemodynamic measurements) and microcirculation (perfusion) has been demonstrated previously.(37) Since PCD was strongly associated with 30-day outcome, monitoring of microcirculation may therefore have additional value for risk stratification as well as for treatment of patients with cardiogenic shock.(38)

Trzeciak et al. recently demonstrated that increased sublingual microcirculatory flow during resuscitation of septic shock was associated with lower SOFA scores at 24 hours.(39) In contrast, we did not find a relationship between changes in sublingual PCD and changes in SOFA score between T0 and T1. Nevertheless, we demonstrated that sublingual PCD at baseline was predictive for recovery from organ failure. In addition, patients who had a PCD ≤ median at T0 as well as at T1 had the highest SOFA scores at T1.

Hasdai and colleagues demonstrated the predictive value of a cold, clammy skin on 30-day mortality in patients with cardiogenic shock complicating acute myocardial infarction.(40) In addition, De Backer et al. reported that the proportion of sublingual perfused capillaries, measured after hospital admission, was higher in patients who survived than in patients who did not survive (64% vs. 43%, $p<0.05$).(36) In our (larger) study, we confirmed these observations and demonstrated that, in patients all having clinical signs of hypoperfusion, sublingual PCD can be used to better define the severity of cardiogenic shock and to refine the prediction of outcome.

Clinical perspectives

These findings raise the question whether sublingual PCD can be used as a therapeutic target at the bedside and, if so, whether interventions directed at improving PCD will be associated with improved outcome. We demonstrated recently that PCD can be improved by pharmacologic therapy (nitroglycerin)(25, 26) as well as by mechanical circulatory support.(29) The current study demonstrates that patients who had a low PCD at baseline which recovered at 24 hours, had a similar prognosis as patients who had a higher PCD at both time points. Taken together, these results suggest that assessment of sublingual PCD by SDF imaging, followed by prompt interventions directed at improving microvascular perfusion, might optimize therapy in order to improve outcome of patients with cardiogenic shock.

Limitations

Several limitations of our study need to be acknowledged. First, measurements of the pulmonary circulation by a pulmonary artery catheter were missing in some patients when the attending clinicians were unwilling to use this monitoring device, even in a research setting. Second, PCD measurements could not be repeated in some patients. Third, we measured patients only after informed consent had been obtained. This implies that, in most cases, it consumed hours before baseline measurements could be performed. Nevertheless, our study clearly demonstrates that in these patients, already being resuscitated, sublingual PCD can be used to identify patients who are at a high risk of dying. Fourth, we used PCD as the marker of microcirculatory perfusion, a software-derived parameter in which microvascular flow and density are combined. This parameter does not take into account the heterogeneity of perfusion, which may

be increased in disease states.(41) However, the problem of heterogeneous blood flow, visualized sublingually as fields of absent or intermittent capillary blood flow, seems to be more specific for septic shock than for cardiogenic shock.(16, 36, 42, 43) Finally, since our study was an observational study, significant correlations, for example between baseline PCD and changes in SOFA score, do not prove causality.

CONCLUSIONS

In conclusion, impaired microcirculation, as assessed by sublingual PCD, is associated with the development of (multi-)organ failure. In addition, this parameter is an independent, strong predictor of outcome. Because of the independent and strong association with prognosis in cardiogenic shock, assessment of sublingual PCD using Sidestream Dark Field imaging should be considered as a simple non-invasive tool to assess outcome in patients with cardiogenic shock. Whether a strategy of improving sublingual PCD will improve survival of patients with cardiogenic shock, should preferably be tested in a future, multi-center randomized trial.

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Chapter 18

Percutaneous left ventricular assist devices vs. intra-aortic balloon pump counterpulsation for treatment of cardiogenic shock: a meta-analysis of controlled trials

Cheng JM, Den Uil CA, Hoeks SE, Van Der Ent M, Jewbali LSD, Van Domburg RT, Serruys PW.

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ABSTRACT

Aims: Studies have compared safety and efficacy of percutaneous left ventricular assist devices (LVADs) with intra-aortic balloon pump (IABP) counterpulsation in patients with cardiogenic shock. We performed a meta-analysis of controlled trials to evaluate potential benefits of percutaneous LVAD on hemodynamics and 30-day survival.

Methods and results: Two independent investigators searched Medline, Embase and Cochrane Central Register of Controlled Trials for all controlled trials using percutaneous LVAD in patients with cardiogenic shock, where after data was extracted using standardized forms. Weighted mean differences (MD) were calculated for cardiac index (CI), mean arterial pressure (MAP) and pulmonary capillary wedge pressure (PCWP). Relative risks (RR) were calculated for 30-day mortality, leg ischemia, bleeding and sepsis. In main analysis, trials were combined using inverse-variance random-effects approach. Two trials evaluated the TandemHeart and a recent trial used the Impella device. After device implantation, percutaneous LVAD patients had higher CI (MD 0.35 l/min/m^2, 95% CI 0.09;0.61), higher MAP (MD 12.8 mm Hg, 95% CI 3.6;22.0) and lower PCWP (MD -5.3 mm Hg, 95% CI -9.4;-1.2) compared to IABP patients. Similar 30-day mortality (RR 1.06, 95% CI 0.68;1.66) was observed using percutaneous LVAD compared to IABP. No significant difference was observed in incidence of leg ischemia (RR 2.59, 95% CI 0.75;8.97) in percutaneous LVAD patients compared to IABP patients. Bleeding (RR 2.35, 95% CI 1.40;3.93) was significantly more observed in TandemHeart patients compared to patients treated with IABP.

Conclusion: Although percutaneous LVAD provides superior hemodynamic support in patients with cardiogenic shock compared to IABP, use of these more powerful devices did not improve early survival. These results do not yet support percutaneous LVAD as first-choice approach in the mechanical management of cardiogenic shock.

INTRODUCTION

Cardiogenic shock is a state of inadequate tissue perfusion due to cardiac dysfunction.(1) Despite the fact that prognosis of patients with cardiogenic shock has improved over time due to aggressive reperfusion strategies, in-hospital mortality from cardiogenic shock remains about 50%.(2-8)

Although recent guidelines supported the use of intra-aortic balloon pump (IABP) counterpulsation as method of first choice for mechanical assistance in cardiogenic shock,(1, 9) the efficacy of routine IABP use adjunctive to primary percutaneous coronary intervention in cardiogenic shock was recently questioned.(10, 11) The recent introduction of percutaneous left ventricular assist devices (LVADs) is very promising since these more powerful devices have the potential to reverse cardiogenic shock and to lower the unacceptably high short-term mortality rates.(12, 13) These LVADs might be a better alternative as compared to IABP in the mechanical treatment of cardiogenic shock.(10, 14) The TandemHeart (TandemHeart, Cardiac Assist, Pittsburgh, PA, USA) is a percutaneous left atrial-to-femoral arterial LVAD, driven by a low-speed centrifugal continuous flow pump.(15) The Impella (Impella LP2.5, Abiomed Europe GmbH, Aachen, Germany) LVAD is a catheter-based, impeller-driven, axial flow pump which pumps blood directly from the left ventricle into the ascending aorta.(13)

Several controlled trials have compared safety and efficacy of these percutaneous LVADs with IABP.(16-18) However, the trials were underpowered to adequately evaluate potential benefit on 30-day outcome. We pooled data from these trials and compared (1) differences in hemodynamic parameters following device implantation, (2) 30-day mortality, and (3) adverse events in patients receiving percutaneous LVAD versus those treated with IABP. Aim of the study was to present an overview on the current status of percutaneous assist devices in the management of cardiogenic shock.

METHODS

Trial inclusion

All controlled trials using percutaneous LVAD in patients with cardiogenic shock were included. Follow-up duration had to be at least 30 days. Using Cochrane Central Register of Controlled Trials, Embase and Medline (Pubmed U.S. National Library of Medicine), we performed a literature search from inception to April 2009 using the following search terms: "heart-assist device" OR "shock, cardiogenic", as well as using the terms separately as text words.(19) A methodological filter was used to limit the results to clinical trials in humans.(19) No language restrictions were used. Two investigators (JMC and CAU) then independently retrieved potentially eligible reports for evaluation. Both investigators

independently examined design, patient population and interventions in the reports. In case of disagreement, this was resolved in consultation with a third reviewer (RTD). Trials without control group and trials using surgical LVADs were excluded. In addition, references of included trials were checked, www.clinicaltrials.gov was searched, conference proceedings were checked and experts were contacted to ensure that no potentially eligible studies were missed. Quality of the reports were assessed in terms of randomization, adequateness of sequence generation, concealment of allocation, blinding and handling of patient attrition.(20, 21) Data was extracted by two independent investigators (JMC and CAU) using standardized forms.

Study outcomes

Thirty-day all-cause mortality was a priori specified as our primary clinical outcome, as this is the most common clinical endpoint in literature on cardiogenic shock. Secondary outcomes were the following prespecified hemodynamic parameters: cardiac index (CI), mean arterial pressure (MAP), and pulmonary capillary wedge pressure (PCWP), all measured within two hours after device implantation. Safety outcomes were chosen a posteriori, and included the following reported device-related adverse events during support: leg ischemia, major bleeding and fever and/or sepsis. Based on incomplete data reported in the studies, we also evaluated occurrence of thrombocytopenia and hemolysis when reported.

Statistical analysis

All data were analyzed with MIX (MIX 1.7, Kitasato Clinical Research Center, Sagamihara, Kanagawa, Japan)(22) and SPSS (SPSS 15.0, SPSS Inc., Chicago, IL, USA) software. Categorical variables were presented in numbers and in percentages. Continuous variables were presented as mean ± standard deviation. For continuous variables reported as median and interquartile range, the mean and standard deviation were estimated. The mean was estimated by the formula $x = (a+2m+b) / 4$ using the values of the median (m), P25 and P75 (a and b, respectively).(23) The estimator $sd = IQR / 1.35$ was used to estimate standard deviation (sd) from the interquartile range (IQR).(21) Weighted mean difference (MD) was used to compare continuous variables and was calculated for the pooled study population. The final results were presented as weighted mean difference with the associated 95% confidence interval. Relative risk (RR) of unadjusted 30-day mortality and adverse events was calculated for each study and for the pooled study population. The final results were presented as unadjusted relative risk with the associated 95% confidence interval. Heterogeneity between trials, defined as variation among the results of individual trials beyond that expected from chance, was assessed with Cochran's Q statistic and I-square statistic. Both inverse variance weighted fixed effect model as well as a random effects model was used for comparison based on mean dif-

ference and relative risk. Conclusions were drawn based on the random effects models. All statistical tests were analyzed two-tailed and a p-value of <0.05 was considered statistically significant.

RESULTS

Three trials met our inclusion criteria and were included in this study (Figure 1). Study characteristics are presented in Table 1. All three trials randomly assigned patients to treatment with percutaneous LVAD or IABP counterpulsation. Two randomized controlled trials compared the TandemHeart device with IABP,(16, 17) and one randomized controlled trial compared the Impella with IABP counterpulsation.(18) One trial reported adequate sequence generation,(16) while two trials omitted description of methods for sequence generation.(17, 18) Methods for allocation concealment were not adequately reported. Complete follow-up was available in all included trials.

Baseline characteristics

Baseline characteristics and baseline hemodynamic parameters of patients included in the randomized controlled trials are presented in Table 2. In the study by Thiele et al. 41 patients with revascularized acute myocardial infarction complicated by cardiogenic shock were included for randomization (21 patients assigned to LVAD and 20 patients

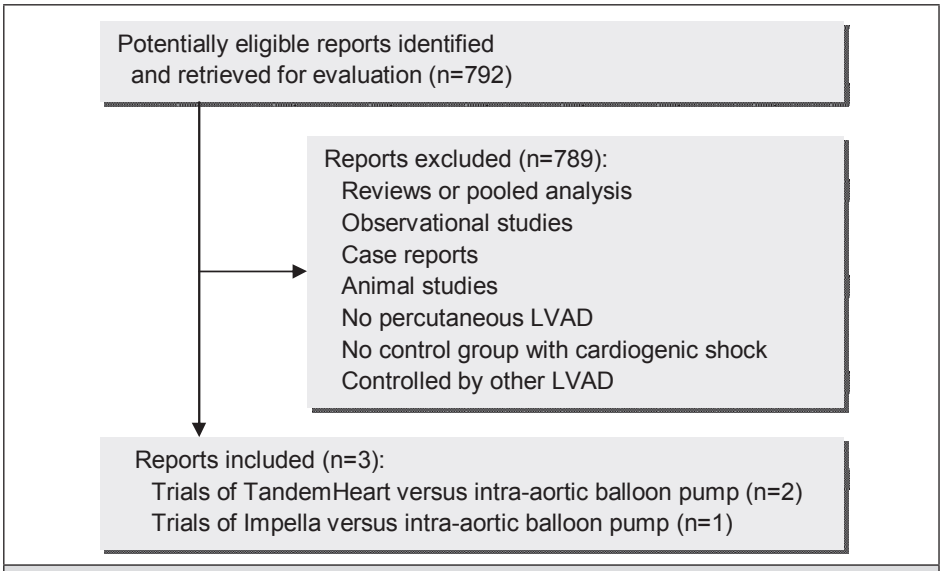

Figure 1. Identification of trials.
LVAD = left ventricular assist device.

Table 1. Study characteristics of included trials

	Thiele et al.(16)	Burkhoff et al.(17)	Seyfarth et al.(18)
Percutaneous LVAD used	TandemHeart	TandemHeart	Impella LP2.5
Control	IABP	IABP	IABP
Total number of patients	41	33	26
Setting	Single-center	Multi-center	Two-center
Inclusion period	2000-2003	2002-2004	2004-2007
Randomization	Yes	Yes	Yes
Sequence generation	Drawing envelopes	Not reported	Not reported
Concealment of allocation	Sealed envelopes*	Not reported	Not reported
Blinding	Not possible	Not possible	Not possible
Handling of patient attrition	Complete follow-up	Complete follow-up	Complete follow-up

* Not reported whether the envelopes were opaque and sequentially numbered.
IABP = intra-aortic balloon pump; LVAD = left ventricular assist device.

Table 2. Baseline characteristics

	Thiele et al.		Burkhoff et al.		Seyfarth et al.	
	LVAD (n=21)	IABP (n=20)	LVAD (n=19)	IABP (n=14)	LVAD (n=13)	IABP (n=13)
Age, years ± SD	63 ± 10	66 ± 10	66 ± 14	60 ± 11	65 ± 10	67 ± 19
Male, n (%)	16 (76)	15 (75)	14 (74)	9 (64)	8 (62)	11 (85)
Hypertension, n (%)	19 (90)	15 (75)			7 (54)	9 (69)
Diabetes mellitus, n (%)	11 (52)	11 (55)			5 (39)	3 (23)
Smoking, n (%)	9 (43)	6 (30)			8 (62)	7 (54)
Hypercholesterolemia, n (%)	11 (52)	9 (45)			8 (62)	7 (54)
Multivesseldisease, n (%)	13 (62)	14 (70)			9 (69)	10 (77)
LVEF, % ± SD	26 ± 9	27 ± 7	19 ± 14	22 ± 9	28 ± 14	31 ± 16
AMI, n (%)	21 (100)	20 (100)	11 (58)	10 (71)	13 (100)	13 (100)
Anterior MI, n (%)	18 (86)	13 (65)			7 (54)	8 (62)
Peak creatine kinase, U/L ± SD	5307 ± 4297	4395 ± 3987			4067 ± 6104	4971 ± 5211
Inotropes or vasopressors, n (%)	21 (100)	20 (100)	19 (100)	14 (100)	11 (84)	12 (92)
Mechanical ventilation, n (%)	20 (95)	20 (100)			12 (92)	12 (92)
Primary PCI, n (%)	20 (95)	19 (95)			12 (92)	12 (92)
Hemodynamics						
CI, l/min/m^2 ± SD	1.8 ± 0.4	1.6 ± 0.5	1.8 ± 0.4	1.8 ± 0.6	1.7 ± 0.5	1.7 ± 0.6
MAP, mm Hg ± SD	62 ± 14	65 ± 13	70 ± 16	67 ± 15	78 ± 16	72 ± 17
PCWP, mm Hg ± SD	20 ± 4	26 ± 7	25 ± 8	28 ± 6	22 ± 8	22 ± 7

AMI = acute myocardial infarction; CI = cardiac index; IABP = intra-aortic balloon pump; LVAD = left ventricular assist device; LVEF = left ventricular ejection fraction; MAP = mean arterial pressure; PCI = percutaneous coronary intervention; PCWP = pulmonary capillary wedge pressure.

assigned to IABP).(16) Burkhoff et al. randomized 33 patients with cardiogenic shock caused by acute myocardial infarction or decompensated chronic heart failure (19 patients assigned to LVAD and 14 patients assigned to IABP).(17) Seyfarth at al randomized 26 patients with acute myocardial infarction complicated by cardiogenic shock (13 patients assigned to LVAD and 13 patients assigned to IABP).(18) In total, 100 patients were included for meta-analysis, of whom 53 patients were treated with LVAD and 47 patients were treated with IABP. Almost all patients were treated with inotropes or vasopressors, mechanical ventilation and percutaneous coronary intervention.

Hemodynamic parameters following device implantation

Hemodynamic parameters measured after device implantation as well as results obtained from both fixed effect models and random effects models showing the pooled mean differences between hemodynamic parameters of LVAD patients compared to IABP patients are presented in Table 3. In the random effects model, patients treated with a percutaneous LVAD had higher CI (MD 0.35 l/min/m^2, 95% CI 0.09 ; 0.61, $p<0.01$), higher MAP (MD 12.8 mm Hg, 95% CI 3.6 ; 22.0, $p<0.01$) and lower PCWP (MD -5.3 mm Hg, 95% CI -9.4 ; -1.2, $p<0.05$) compared to patients treated with IABP. (Figure 2)

30-day mortality

Reported absolute thirty-day all-cause mortality as well as results obtained from both fixed effect model and random effects model showing the relative risk are presented in Table 3. In the pooled study population, 24 patients (45%) treated with LVAD and 20 patients (43%) treated with IABP did not survive 30 days of follow-up ($p=0.80$). The pooled estimate of the relative risk revealed no significant difference in 30-day mortality using percutaneous LVAD compared to IABP (RR 1.06, 95% CI 0.68 ; 1.66). (Figure 3)

Adverse events

Reported adverse events as well as results obtained from both fixed effect models and random effects models showing the relative risk are presented in Table 3. Using a random effects model, similar incidence rates of leg ischemia were observed using percutaneous LVAD as compared to IABP (RR 2.59, 95% CI 0.75 ; 8.97, $p=0.13$). (Figure 4) Bleeding (RR 2.35, 95% CI 1.40 ; 3.93, $p<0.01$) was more frequently reported as a complication related to the TandemHeart. Furthermore, Thiele et al. reported that fresh frozen plasma ($p<0.01$) and platelets ($p<0.05$) were more often required in the TandemHeart group. However, Burkhoff et al. found no significant difference in thrombocytopenia, but these investigators did find a trend toward more hemolysis with higher peak values in plasma free hemoglobin in patients treated with the Tandemheart ($p=0.10$).

Table 3. Meta-analysis of outcomes

	Thiele et al.		Burkhoff et al.		Seyfarth et al.		Pooled (fixed effect model)			Pooled (random effects model)		
	LVAD (n=21)	IABP (n=20)	LVAD (n=19)	IABP (n=14)	LVAD (n=13)	IABP (n=13)	Mean difference / Relative Risk		*p*	Mean difference / Relative Risk		*p*
Hemodynamics												
CI, l/min/m^2 ± SD	2.3 ± 0.6	1.8 ± 0.4	2.2 ± 0.6	2.1 ± 0.2	2.2 ± 0.6	1.8 ± 0.7	0.35	(0.14 ; 0.55)	<0.001	0.35	(0.09 ; 0.61)	<0.01
MAP, mm Hg ± SD	76 ± 10	70 ± 16	91 ± 16	72 ± 12	87 ± 18	71 ± 22	12.1	(6.3 ; 17.9)	<0.001	12.8	(3.6 ; 22.0)	<0.01
PCWP, mm Hg ± SD	16 ± 5	22 ± 7	16 ± 4	25 ± 3	19 ± 5	20 ± 6	-6.2	(-8.0 ; -4.3)	<0.001	-5.3	(-9.4 ; -1.2)	<0.05
Clinical outcome												
30-day mortality, n (%)	9 (43)	9 (45)	9 (47)	5 (36)	6 (46)	6 (46)	1.06	(0.68 ; 1.66)	0.80	1.06	(0.68 ; 1.66)	0.80
Reported adverse events												
Leg ischemia, n (%)	7 (33)	0 (0)	4 (21)	2 (14)	1 (8)	0 (0)	2.59	(0.75 ; 8.97)	0.13	2.59	(0.75 ; 8.97)	0.13
Bleeding, n (%)	19 (90)	8 (40)	8 (42)	2 (14)			2.35	(1.40 ; 3.93)	<0.01	2.35	(1.40 ; 3.93)	<0.01
Fever of sepsis, n (%)	17 (81)	10 (50)	4 (21)	5 (36)			1.38	(0.88 ; 2.15)	0.16	1.11	(0.43 ; 2.90)	0.83

CI = cardiac index; IABP = intra-aortic balloon pump; LVAD = left ventricular assist device; MAP = mean arterial pressure; PCWP = pulmonary capillary wedge pressure.

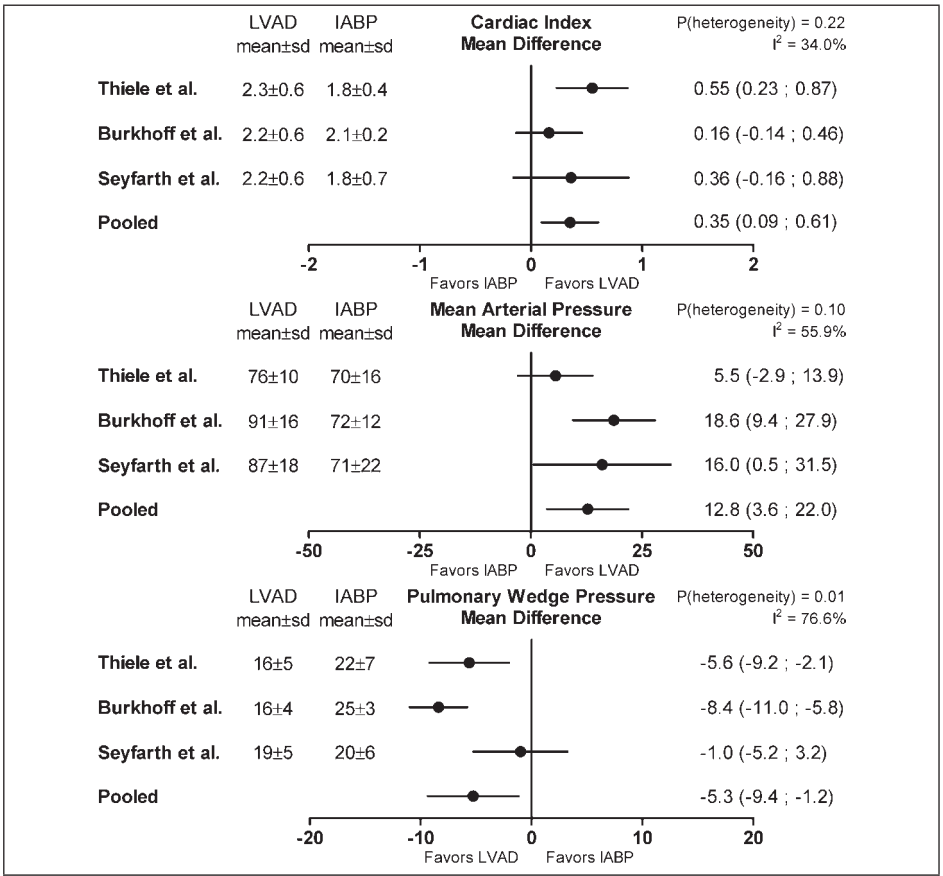

Figure 2. Meta-analysis showing the mean difference in hemodynamic parameters with use of percutaneous left ventricular assist devices.
Random effects models were used for meta-analysis. Weighted mean differences with 95% confidence intervals are presented on the right of the figure. IABP = intra-aortic balloon pump; LVAD = left ventricular assist device.

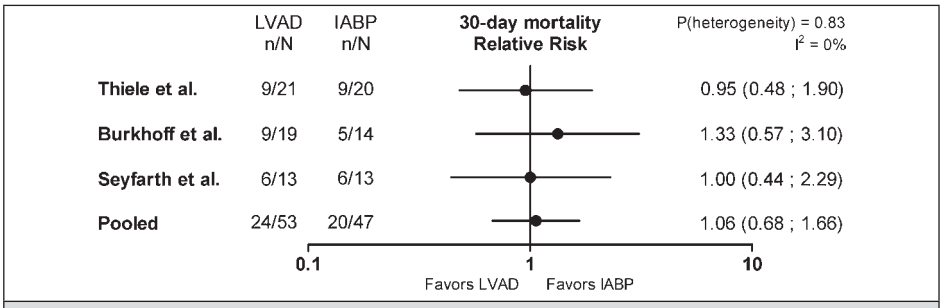

Figure 3. Meta-analysis showing the relative risk of crude 30-day mortality with use of percutaneous left ventricular assist devices.
Random effects model was used for meta-analysis. Relative risks with 95% confidence intervals are presented on the right of the figure. IABP = intra-aortic balloon pump; LVAD = left ventricular assist device.

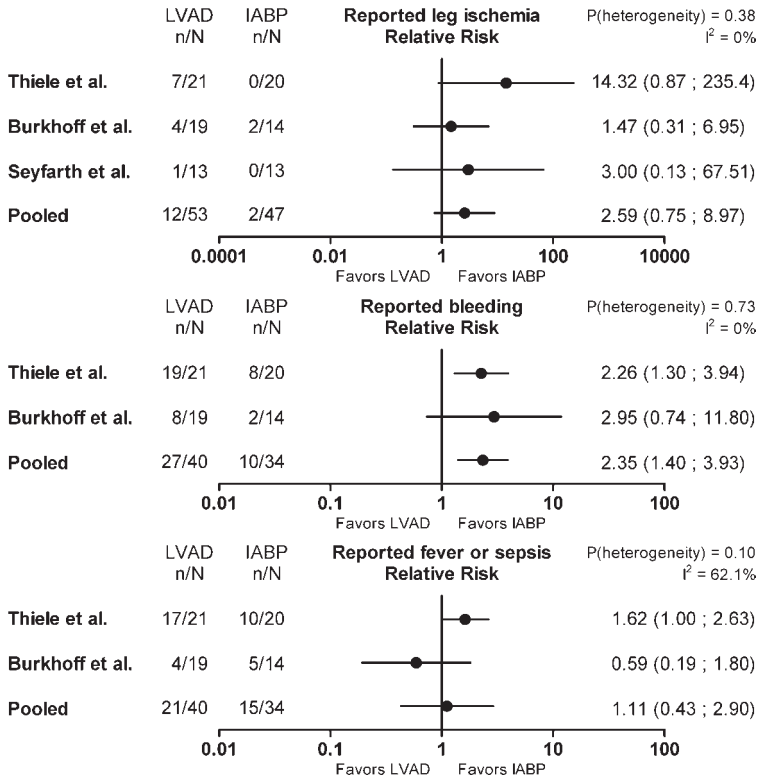

Figure 4. Meta-analysis showing the relative risk of adverse events with use of percutaneous left ventricular assist devices.

Random effects models were used for meta-analysis. Relative risks with 95% confidence intervals are presented on the right of the figure.

IABP = intra-aortic balloon pump; LVAD = left ventricular assist device.

No reports were found on Impella-related incidence of bleeding and fever and/or sepsis. However, a trend was reported for more packed red blood cells (Impella 2.6 ± 2.7 units versus IABP 1.2 ± 1.9 units, p=0.2) and fresh frozen plasma (Impella 1.8 ± 2.5 units versus IABP 1.0 ± 1.7 units, p= 0.4) administered to Impella patients. Hemolysis was assessed by measurements of free hemoglobin, which was significantly higher in Impella patients ($p<0.05$).

DISCUSSION

This is the first meta-analysis of controlled trials comparing percutaneous LVAD with IABP, presenting a survey of available data. Our main findings were that although use of percutaneous LVAD resulted in a better hemodynamic profile compared to IABP coun-

terpulsation, this did not translate into improved 30-day survival. Moreover, patients treated with a percutaneous LVAD tended to have a higher incidence of leg ischemia and device-related bleeding.

The main limitation of an IABP is the lack of active cardiac support: the IABP requires a certain residual level of left ventricular function. As an alternative, percutaneous LVADs are promising devices since these provide active circulatory support. This meta-analysis indeed confirms that a percutaneous LVAD is a more powerful device than IABP, which is clearly reflected by a better hemodynamic profile post implantation.

Although both types of percutaneous LVADs improved the hemodynamic profile, it is disappointing that both devices did not improve 30-day outcome as compared to current routine treatment including IABP. Besides, it is important to note that both percutaneous LVADs are currently about 10 times as expensive as an IABP catheter.

We reported similar complication rates associated with both types of percutaneous LVADs as compared to IABP. However, it might be possible that the Impella is a safer device than the TandemHeart due to its smaller catheter size, potentially resulting in a lower incidence of leg ischemia or groin bleeding,(24) although this is not clearly demonstrated by this meta-analysis. Seventeen French cannulas were used in TandemHeart patients and 13 French sheaths were used in Impella patients, whereas most IABPs are currently introduced using 8 French sheaths. Although the way of vascular closure was not consistently reported in the trials, this could also be a factor involved in the development of vascular complications. Whether hemolysis is a clinically significant problem associated with Impella use, has to be investigated further.

Some limitations of our meta-analysis need to be acknowledged. First, we compared different types of percutaneous LVADs (i.e. TandemHeart and Impella) with IABP. However, there was no heterogeneity between TandemHeart and Impella studies in 30-day mortality and in most secondary study outcomes. Because the small number of trials included could possibly lead to a type II error of the heterogeneity test, all conclusions were based on results obtained from random effects models. Second, the number of patients included in this meta-analysis was small. However, we included all available trials and we did not even observe a trend in a reduced 30-day mortality rate associated with LVAD use. The results from this meta-analysis suggest that potential benefit of percutaneous LVADs on 30-day survival might be very limited. Due to the small sample size, there is a probability of missing a clinically meaningful benefit if one exists (type II error).(25) However, given a total sample size of 100 patients, an observed p-value (α) of 0.80 and a presumed effect size of at least 10% (event rate of 45% in IABP patients and 40% in percutaneous LVAD patients), post-hoc analysis showed that the probability for type II error (β) does not exceed 12%. A third limitation was that we did not have access to individual patient data. It may be very well possible that subgroups of cardiogenic shock patients might benefit from percutaneous LVAD therapy, but we could not perform

these analyses given the limited number of patients included in the currently available reports. A final limitation was that the included trials were not described in sufficient detail to judge adequateness of randomization, so that we were not able to exclude the potential risk of bias in these trials.

Hopefully, further technical improvements on percutaneous LVAD systems, together with enhanced experience with these devices, will improve prognosis of cardiogenic shock patients in the future. A larger, adequately powered, randomized controlled trial using the Impella device is necessary to provide more definite information about potential benefit on 30-day survival. Some investigators have shown the feasibility of introduction of surgical LVADs in patients with acute myocardial infarction complicated by cardiogenic shock.(26) A major problem of implanting a surgical LVAD includes apical cannulation in infarcted myocardium. The recent development of a micropump, inserted via a mini-thoracotomy and providing substantial left ventricular support, is very promising, but has to be investigated in larger studies and in the setting of cardiogenic shock.(27)

In conclusion, in patients presenting with cardiogenic shock, the use of a percutaneous LVAD provides superior hemodynamic support compared to use of IABP. However, a better hemodynamic profile associated with percutaneous LVAD use did not result into a reduced 30-day mortality rate. Furthermore, a higher rate of adverse events was encountered by the higher invasive nature of LVAD, especially of the TandemHeart device. Larger randomized controlled trials using the Impella device are needed to better evaluate clinical outcome and adverse events. Until now, we cannot recommend to replace IABP counterpulsation by the more powerful percutaneous LVAD for the treatment of cardiogenic shock patients who do not respond sufficiently to pharmacologic therapy.

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Chapter 19

Impact of intra-aortic balloon pump support initiated before versus after primary percutaneous coronary intervention in patients with cardiogenic shock from acute myocardial infarction

Cheng JM, Van Leeuwen MAH, de Boer SPM, Tjong Joe Wai MCG, den Uil CA, Jewbali LSD, Van Geuns RJ, Kardys I, van Domburg RT, Boersma E, Zijlstra F, Akkerhuis KM.

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ABSTRACT

Background: Little evidence is available on the optimal sequence of intra-aortic balloon pump (IABP) support initiation and primary percutaneous coronary intervention (PCI) in patients who present with cardiogenic shock from ST-elevation myocardial infarction (STEMI). The aim of this study was to evaluate the order of IABP insertion and primary PCI and its association with infarct size and mortality.

Methods: A series of 173 consecutive patients admitted with cardiogenic shock from STEMI and treated with primary PCI and IABP between 2000 and 2009 were included. The order of IABP insertion and primary PCI was left at the discretion of the interventional cardiologist.

Results: All baseline characteristics were similar in patients who first received IABP (n=87) and patients who received IABP directly after PCI (n=86). In these two groups, cumulative 30-day mortality was 44% and 37% respectively (p=0.39). Median peak serum creatine kinase (CK) concentration was 5692 U/L and 4034 U/L respectively (p=0.048). In multivariable analysis, IABP insertion before PCI was independently associated with higher CK levels (p=0.046). In patients who survived 30-days, IABP insertion before PCI was not associated with late mortality evaluated at five years of follow-up (HR1.5, 95%CI 0.7-3.3; p=0.34).

Conclusions: Early IABP insertion before primary PCI might be associated with higher peak CK levels, indicating a larger infarct size. A possible explanation may be the increased reperfusion delay. Our study suggests that early reperfusion could have priority over routine early IABP insertion in STEMI patients with cardiogenic shock. Randomized studies are needed to determine the optimal timing of IABP insertion relative to primary PCI.

INTRODUCTION

Cardiogenic shock complicating ST-segment elevation myocardial infarction (STEMI) remains the leading cause of death in this patient population, despite improvements in therapy over the last decade including primary PCI.(1-2) The in-hospital mortality rate has been reported to be as high as 50%.(1-2) Intra-aortic balloon pump (IABP) counterpulsation is the most widely used method of mechanical support in cardiogenic shock.(3-5) Although the efficacy of IABP adjunctive to primary percutaneous coronary intervention (PCI) has been questioned, IABP remains the method of first choice for mechanical assistance in patients with cardiogenic shock who do not respond adequately to standard pharmacological treatment.(6-7)

With regard to the sequence of both treatments, current guidelines recommend insertion of an IABP before primary PCI.(3-4) However, there is little evidence supporting this recommendation.(8) Hypothetically, IABP counterpulsation may provide additional hemodynamic support during the procedure when it is inserted prior to percutaneous coronary revascularization. On the other hand, each additional minute in delay to the actual reperfusion of the occluded coronary artery, including the time needed for IABP insertion, may result in decreased myocardial salvage from primary PCI, and thus increased myocardial infarct size.(9-10) Moreover, early revascularization is the only treatment proven to decrease mortality rates in patients with acute myocardial infarction complicated by cardiogenic shock.(11-12) Therefore, the aim of this study was to evaluate the order of IABP insertion and primary PCI and its association with infarct size, 30-day and late mortality in a series of 173 STEMI patients presenting with cardiogenic shock in our hospital.

METHODS

Study population

Between January 2000 and December 2009, all consecutive STEMI patients presenting with cardiogenic shock who were treated with IABP and primary PCI at the Erasmus MC in Rotterdam, the Netherlands, were included. In order to evaluate the effects associated with the relative timing of IABP insertion and primary PCI, we only included patients who presented with cardiogenic shock at time of admission to our hospital and excluded those who developed cardiogenic shock during primary PCI or during hospital stay.

Our center is a tertiary referral center for primary PCI, mechanical support for cardiogenic shock (e.g. IABP counterpulsation) and heart transplantation. During the inclusion period, about 450 STEMI patients were admitted to our center annually. STEMI was defined as acute myocardial infarction with clinical symptoms of ischemia, persistent

(>20 min) ST-segment elevation in at least 2 contiguous precordial leads or at least 2 adjacent limb leads by ECG, and a diagnostic rise in cardiac markers during the hospitalization period.(4) Cardiogenic shock was defined as systolic blood pressure <90 mm Hg due to cardiac insufficiency with clinical signs of hypoperfusion (cold extremities, oliguria, altered mental state etc.), not responsive to fluid resuscitation.(4) In our center, IABP counterpulsation was the method of first choice for mechanical assistance in the patients with cardiogenic shock who did not adequately respond to standard pharmacologic treatment including inotropics. In our hospital, consensus was reached that IABP insertion should be withheld only when insertion was technically not feasible (e.g. because of severe atherosclerotic peripheral artery disease, aortic disease or aortic insufficiency), as well as in patients judged to have a definite fatal short-term prognosis because of concomitant disease. Ethics committee approval or informed consent was not required for this study according to Dutch Ethical Review Board regulations.

Patient management

Patient management was in accordance with the STEMI guidelines of the European Society of Cardiology (ESC).(3-4,13) As an exception, consensus was reached in our hospital that there was no clear evidence on the optimal timing of IABP insertion relative to primary PCI. Therefore, the order of IABP insertion and primary PCI was left at the discretion of the interventional cardiologist. When the operator decided to insert the IABP first, this was done in the catheterization laboratory before angiography and PCI. When the operator decided to perform primary PCI first, the IABP was inserted directly following the PCI procedure in the catheterization laboratory. In all study patients, Arrow (Arrow Corp., Reading, PA, USA) 8 French IABP catheters were used.

Inotropic agents used in our hospital were catecholamines (dobutamine, dopamine, and/or norepinephrine) and phosphodiesterase inhibitors (enoximone). During the study period, primary PCI was the standard treatment of STEMI. Patients received an aspirin and clopidogrel (300 to 600 mg) loading dose prior to primary PCI. Primary PCI was performed using bare metal stents from January 2000 until April 2002, sirolimus eluting stents from April 2002 until March 2003, paclitaxel eluting stents from March 2003 until March 2007 and everolimus eluting stents from March 2007 to December 2009. After the procedure, all patients were advised to remain on aspirin (>80mg/day) indefinitely. Patients were treated with Clopidogrel (75mg/day) for at least one month for those treated with bare metal stents, at least 3 months for patients treated with sirolimus eluting stents, at least 6 months for patients treated with paclitaxel eluting stents and at least 12 months for patients treated with everolimus eluting stents. Use of periprocedural glycoprotein IIb/IIIa antagonists was left at the discretion of the interventional cardiologist.

Study endpoints

Primary study endpoints were enzymatic infarct size measured by peak serum creatine kinase (CK) concentration and 30-day all-cause mortality. Serum CK was measured every 6 hours after hospital admission until a clear peak of CK concentration had been reached. Other serum biomarkers of myocardial injury were not routinely measured during the whole inclusion period. Secondary study endpoints included peak serum CK-MB activity level, CK-MB mass concentration, troponin-T concentration and residual changes on the first 12-lead electrocardiogram (ECG) that was required after PCI (<24 hours). Serum CK-MB activity levels were available in patients admitted before April 2006 (n=99), while CK-MB mass measurements were available in patients admitted after April 2006 (n=74). Serum troponin-T concentrations were available in patients admitted after January 2001 (n=160). Infarct related pathologic Q-wave formation and cumulative residual ST-deviation in all ECG leads were analyzed according to the universal ESC/American College of Cardiology (ACC)/American Heart Association (AHA) definitions.(14) Furthermore, we performed a landmark analysis in patients who survived the first 30 days after primary PCI (30-day survivors) to evaluate late all-cause mortality at 5 years of follow-up.

Data collection and follow-up

Baseline, clinical and procedural characteristics were prospectively entered into digital patients records. These data were retrospectively retrieved and recorded in a dedicated database. The reported blood pressures were measured at arrival in the catheterization laboratory, thus before IABP insertion and primary PCI. For patients admitted from January 2000 until June 2005, the order of IABP insertion and primary PCI was acquired retrospectively from patient medical records and primary PCI reports. After July 2005, the order of IABP insertion and primary PCI was entered prospectively in a dedicated database. In March 2012, vital status of all patients was acquired from municipal civil registries.

Statistical analysis

All data were analyzed with SPSS software (SPSS 17.0, SPSS Inc., Chicago, IL, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant. Normally distributed continuous variables were compared by Student *t* test and are presented as mean ± standard deviation. Non-parametric continuous variables were compared by Mann-Whitney U test and are presented as median and interquartile range (IQR). Categorical variables were compared by chi-square test or Fisher's exact test, when appropriate, and are presented in percentages. Univariable and multivariable linear regression analyses were performed to evaluate the relationship between timing of IABP insertion with peak CK concentration. In multivariable analyses, the covariates age, sex, history of myocardial infarction, out-of-hospital cardiac arrest, mechanical ventilation,

systolic blood pressure, location of myocardial infarction and left main culprit lesion were a priori chosen for inclusion in the model, since these clinical characteristics are well known to be associated with infarct size and/or clinical outcome. The final results are presented as unadjusted and adjusted beta (β) ± standard error (SE).

Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Cumulative mortality was estimated according to the Kaplan-Meier method. Kaplan-Meier survival curves were compared by log-rank test. Univariable and multivariable logistic regression analyses were performed to evaluate the relationship between timing of IABP insertion with 30-day mortality. In patients who survived the first 30 days, multivariate Cox proportional hazards regression analyses were performed to evaluate the relationship between timing of IABP and long-term all-cause mortality. The final results are presented as unadjusted and adjusted odds ratios (OR), and respectively as unadjusted and adjusted hazard ratios (HR), both with the associated 95% confidence intervals (95% CI).

RESULTS

Patient characteristics

During the inclusion period, 4352 STEMI patients underwent primary PCI in our center (Figure 1). In our center, the median symptom-to-balloon time for STEMI patients in that time period was 166 [IQR 110-275] minutes. A total of 291 patients were treated with IABP counterpulsation because of cardiogenic shock. After exclusion of patients who did not present with cardiogenic shock at the time of admission to our hospital, 173 patients were included in this study. The baseline and procedural characteristics of patients who received IABP before primary PCI (n=87) and patients who received IABP after primary PCI (n=86) are presented in Table 1. Mean age of the total study population was 64 ± 12 years and 79% were men. No significant differences were present in baseline characteristics among the two groups. One-quarter of the patients experienced an out-of-hospital cardiac arrest. Median serum lactate level was 3.0 mmol/l [IQR 1.9-6.5] in patients who received IABP before primary PCI and 3.2 mmol/l [IQR 1.8-6.0] in patients who received IABP after primary PCI (p=0.97). At the time of hospital admission, serum CK (p=0.58), CK-MB activity (p=0.64), CK-MB mass (p=0.25) and troponin-T (p=0.79) concentrations were similar among the two groups. In 66% of the patients, the infarction was located anteroseptal. Rupture of the septal wall was documented in 2 patients (both received IABP after PCI), while 2 other patients had a rupture of the papillary muscle (1 in each group). Primary PCI was performed under mechanical ventilation in 47% of the patients. The culprit lesion was found in the left anterior descending (LAD) artery in 47% of the patients, and in the left main (LM) coronary artery in 14% of the patients.

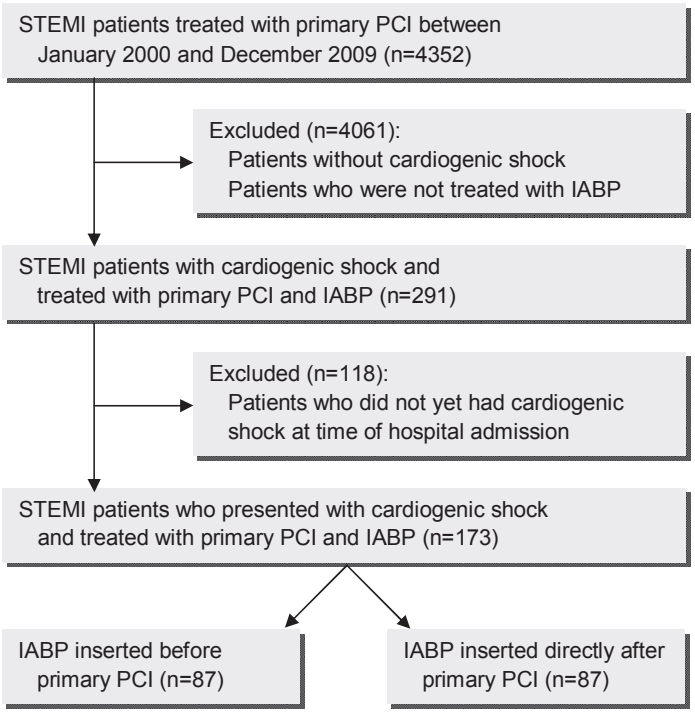

Figure 1. Patient inclusion

Table 1. Patient characteristics

	Total (n=173)	IABP before PCI (n=87)	IABP after PCI (n=86)	*P*-value
Baseline characteristics				
Age, years ± SD	64 ± 12	65 ± 11	64 ± 12	0.72
Men, %	79	79	78	0.82
Diabetes Mellitus, %	17	17	16	0.87
Hypertension, %	29	30	28	0.77
Hypercholesterolemia, %	52	56	48	0.26
Smoking, %	27	24	29	0.46
Positive family history, %	11	13	9	0.48
Previous MI, %	24	30	19	0.084
Previous PCI, %	9	8	11	0.58
Previous CABG, %	5	6	5	0.75
Systolic blood pressure, mmHg ± SD	77 ± 14	75 ± 15	79 ± 13	0.11
Heart rate, bpm ± SD	93 ± 29	92 ± 25	94 ± 33	0.61
Location of myocardial infarction				0.71
Anterior/septal, %	66	68	65	
Inferior/posterior, %	34	32	35	

Table 1. Patient characteristics (continued)				
	Total (n=173)	IABP before PCI (n=87)	IABP after PCI (n=86)	*P*-value
Out-of-hospital cardiac arrest, %	25	23	27	0.57
Mechanical ventilation, %	47	49	44	0.49
Defibrillation, %	25	24	26	0.83
Serum lactate, mmol/l [IQR]	3.1 [1.8-6.2]	3.0 [1.9-6.5]	3.2 [1.8-6.0]	0.97
Cardiac biomarkers at admission				
CK, U/l [IQR]	498 [135-2144]	361 [120-2199]	652 [142-1930]	0.58
CK-MB activity*, U/l [IQR]	169 [69-389]	154 [68-360]	174 [67-433]	0.64
CK-MB mass*, ug/l [IQR]	9.3 [5.0-67.5]	8.6 [4.8-61.1]	12.6 [5.1-75.3]	0.25
Troponin-T*, ug/l [IQR]	0.56 [0.04-2.76]	0.54 [0.07-3.01]	0.64 [0.04-2.54]	0.79
Procedural characteristics				
Culprit lesion				0.48
Left main, %	14	16	12	
Left anterior descending, %	47	51	44	
Left circumflex, %	13	12	14	
Right, %	22	16	27	
Sapheinous vein graft, %	4	5	3	
Multi vessel treatment, %	13	17	9	0.12
Glycoprotein IIb/IIIa inhibitor, %	20	20	20	0.97

* CK-MB activity levels were available in patients admitted before April 2006 (n=99); CK-MB mass levels were available in patients admitted after April 2006 (n=74); troponin-T levels were available in patients admitted after January 2001 (n=160).

Infarct size

Patients who received IABP before primary PCI had a higher median peak CK level compared to patients who received IABP after primary PCI (5692 U/l, IQR 3326-9488 versus 4034 U/l, IQR 2270-7123; p=0.048) (Figure 2). This association remained statistically significant in the multivariable analysis (β 1864 ± 925; p=0.046) (Table 2). Median peak CK-MB activity level was 632 U/l [IQR 369-887] in patients who received IABP before PCI versus 488 U/l [IQR 338-769] in patients who received IABP after PCI (p=0.26). Median peak CK-MB mass concentration was 305 ug/l [IQR 114-595] versus 239 ug/l [IQR108-528] respectively (p=0.70). Median peak troponin-T concentration was 12.1 mmol/l [IQR 6.13-19.5] versus 9.26 mmol/l [IQR 4.52-16.0] respectively (p=0.046).

Post-procedural ECGs (<24 hours post-PCI) were available in 95 patients (55%). Residual ECG changes were evaluated in patients who received IABP before primary PCI and in patients who received IABP after PCI. The median number of ECG leads with infarct related pathologic Q wave formation was not statistically different in the two groups (3, IQR 2-3 versus 2, IQR 0-3 respectively; Mann-Whitney U p=0.38). The median cumulative residual ST-deviation was not statistically significant in the two groups (7mm, IQR 4-13 versus 7mm, IQR 3-11 respectively; Mann-Whitney U p=0.41).

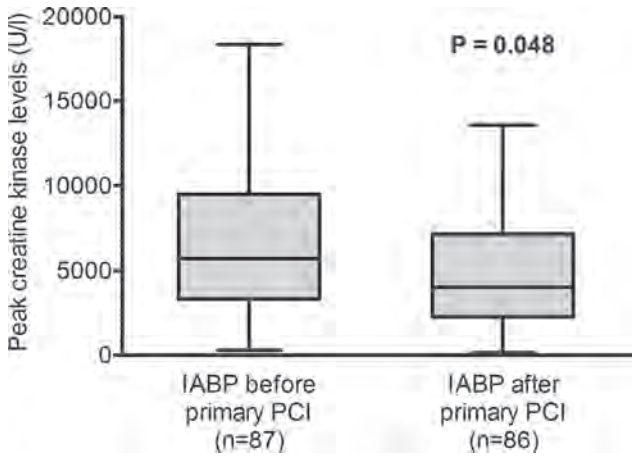

Figure 2. Peak creatine kinase levels
Peak creatine kinase (CK) concentrations in patients who received IABP before primary PCI (median 5692 U/l; IQR 3326-9488) compared to those who received IABP directly after PCI (median 4034 U/l; IQR 2270-7123).

Table 2. Clinical outcomes

	IABP before PCI compared to after PCI	*P*-value
Enzymatic infarct size (peak CK concentration)		
Univariate	β 1951 ± 978	0.048
Multivariate*	β 1864 ± 925	0.046
30-day mortality		
Univariate	OR 1.3 (0.7-2.4)	0.39
Multivariate*	OR 1.0 (0.5-1.9)	0.89
5-year mortality of patients who survived the first 30 days		
Univariate	HR 1.7 (0.8-3.5)	0.16
Multivariate*	HR 1.5 (0.7-3.3)	0.34

Clinical outcomes of patients who received IABP before primary PCI are compared to that of patients who received IABP directly after PCI. Linear regression analyses were performed to evaluate peak CK concentration and are presented as unadjusted and adjusted beta (β) ± standard error (SE). Logistic regression analyses and Cox regression analyses were performed to evaluate mortality are presented as unadjusted and adjusted odds ratios (OR), and respectively as unadjusted and adjusted hazard ratios (HR), both with the associated 95% confidence intervals (95% CI).
* In multivariable analyses, the variables age, sex, history of MI, out-of-hospital cardiac arrest, mechanical ventilation, systolic blood pressure, location of MI and culprit lesion in left main coronary artery were entered into the model.

30-day mortality

Vital status was acquired for all included patients. Two patients (1%) were lost during follow-up. Median follow-up duration was 5.7 years and ranged from 2.3 to 12.2 years. In the total study population, cumulative 30-day mortality rate was 40%. Kaplan-Meier 30-day mortality was 44% in patients who received IABP before primary PCI compared to 37% in patients who received IABP after primary PCI (logrank p=0.39) (Figure 3). After adjustment in multivariable analysis, the timing of IABP insertion relative to primary PCI was not associated with 30-day mortality (OR 1.0, 95% CI 0.5-1.9; p=0.89) (Table 2).

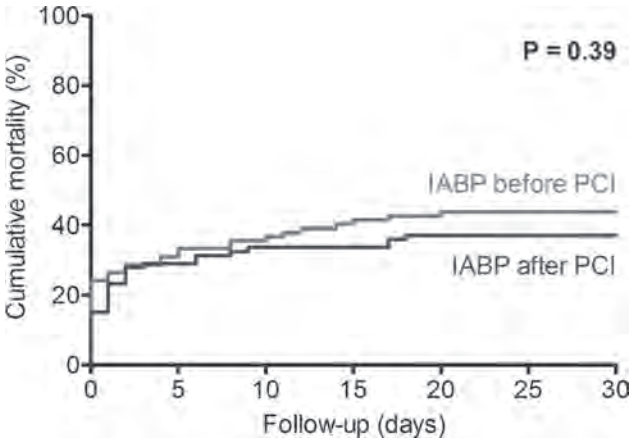

Figure 3. Kaplan-Meier cumulative 30-day mortality

Late mortality

Overall cumulative 1-, 3-, and 5-year mortality rates were 46%, 53% and 59% respectively. Late mortality was analyzed in patients who survived the first 30-days after primary PCI (n=103). In these 30-day survivors, annual mortality rate was 8% for patients who received IABP before primary PCI compared to 5% for patients who received IABP after primary PCI (p=0.16) (Figure 4). After adjustment in multivariable analysis, the order of IABP insertion and primary PCI was not associated with late mortality (HR 1.5, 95% CI 0.7-3.3; p=0.34).

IABP related complications

Limb ischemia was documented in 4 patients (3 patients received IABP before primary PCI and 1 patient received IABP after PCI). In these patients, the limb ischemia recovered spontaneously after removal of the IABP. None of the patients had a major bleeding at the IABP insertion site that required blood transfusion. Vascular surgery for femoral artery pseudo-aneurysm was required in 1 patient who received IABP after primary PCI.

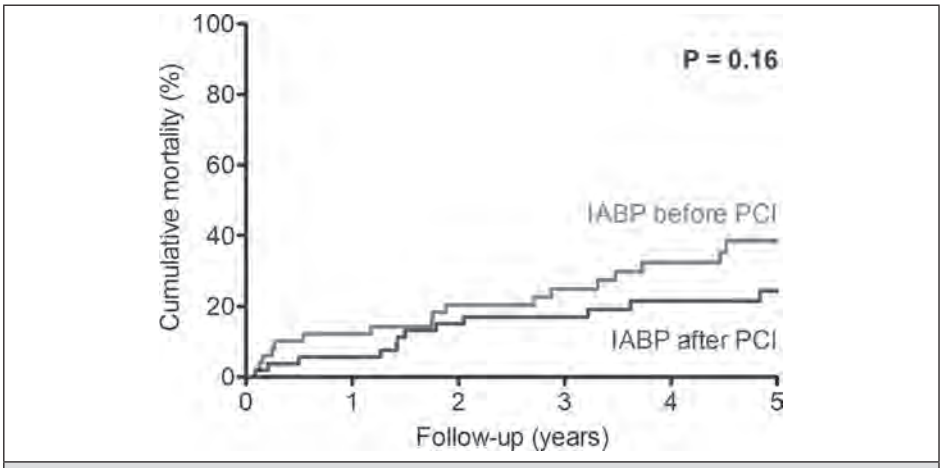

Figure 4. Late mortality in patients who have survived the first 30-days

DISCUSSION

This study compares clinical outcomes of IABP insertion before versus after primary PCI in STEMI patients presenting with cardiogenic shock. Our main finding is that IABP insertion before primary PCI is associated with higher serum peak CK concentration, indicating a larger infarct size, compared to IABP insertion directly after percutaneous coronary revascularization. The order of IABP insertion and primary PCI was not associated with 30-day and late mortality.

The current ESC guidelines on STEMI recommend that the IABP should be inserted before primary PCI, particularly in those patients with cardiogenic shock.(3) Based on a small population of 48 cardiogenic shock patients, Abdel-Wahab et al. reported that patients who underwent primary PCI assisted by IABP had a more favorable in-hospital survival than patients who received the IABP after primary PCI.(8) In contrast, efficacy of IABP counterpulsation adjunctive to primary PCI has been questioned, while rapid reperfusion therapy is the only proven treatment for STEMI complicated by cardiogenic shock.(6,11-12)

In our hospital, consensus was reached that there was no clear evidence on the optimal timing of IABP insertion relative to primary PCI. Therefore, the order of IABP insertion and primary PCI is left at discretion of the interventional cardiologist in our hospital. During the inclusion period, about half of the patients first received IABP, while the other half of the patients first received primary PCI. Noticeable, the baseline characteristics were similar in the 2 groups. This suggests that the chosen sequence of the two treatment modalities was not based on these patient characteristics, but more likely on the preference of the operator.

In this study, peak serum CK level was used as primary endpoint to estimate infarct size. Peak CK level has been shown to correlate well with infarct size measured with single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI).(15-16) The area under the time-CK concentration could not be acquired for all study patients, and therefore has not been evaluated in this study. Serum peak CK-MB and troponin-T were used as secondary endpoints, because the data on these biomarkers were not complete for all included patients. Patients who first received IABP had higher peak CK, peak CK-MB and peak troponin-T levels compared to patients who received IABP directly after primary PCI, although this was not statistically significant for CK-MB activity and CK-MB mass.

A possible explanation of the observed larger infarct size when patients received an IABP prior to primary PCI is that the beneficial effects with IABP counterpulsation are offset by the increased reperfusion delay associated with the time needed for IABP insertion. Unfortunately, data on the length of this delay was not available in this study. A previous randomized study in STEMI patients without cardiogenic shock reported an additional delay of approximately 10 minutes in patients who received an IABP before primary PCI compared to those who did not receive an IABP prior to PCI.(17) Although this delay of approximately 10 minutes seems small, it has been well shown that increased time to reperfusion markedly increases the extent of irreversible myocardial damage, especially in the first hours after symptom onset.(9-10) Considering the median symptom-to-balloon time of 166 minutes (IQR 110-275) in the study period, it is conceivable that an additional delay of 10 minutes may have had clinical significance in this respect (i.e. larger infarct size). Moreover, it should also be considered that the culprit lesion was often found in a proximal segment of the coronary arteries (e.g. 14% in the left main) so that large amounts of myocardium were at risk. A significant amount of myocardium may probably be salvaged if reperfusion occurs as soon as possible.

The Counterpulsation to Reduce Infarct Size Pre-PCI Acute Myocardial Infarction (CRISP AMI) trial randomized patients with acute anterior STEMI without cardiogenic shock to initial IABP counterpulsation before primary PCI versus primary PCI alone.(17) This study showed that early IABP insertion before primary PCI did not result in a reduced infarct size compared to primary PCI alone. Moreover, there even was a trend towards a larger infarct size in patients who received an IABP before primary PCI (p=0.06). These results are in line with our findings.

Previously, Abdel-Wahab et al. compared in-hospital mortality with IABP insertion before versus after primary PCI in 48 STEMI patients with cardiogenic shock.(8) They found that patients who underwent IABP-assisted PCI had lower in-hospital mortality (19%) than patients who received IABP after primary PCI (59%). In our study, no association was found between the order of IABP insertion and primary PCI with 30-day mortality. Some differences in study population and study methods may explain these contrary

results. Firstly, we only included patients who presented with cardiogenic shock at time of hospital admission and excluded those who developed cardiogenic shock during primary PCI or during hospital stay. Secondly, in patients who received primary PCI first, there was a difference in the timing of IABP insertion. In our study, the IABP was inserted directly after the primary PCI, whereas Abdel-Wahab et al. inserted the IABP within 24 hours in case hemodynamic stabilization did not occur.

The IABP-SHOCK II trial randomly assigned 600 patients with acute myocardial infarction and cardiogenic shock for whom an early revascularization strategy was planned to IABP or no IABP support.(18) They found that the use of IABP did not significantly reduced 30-day mortality. Therefore, the class I recommendation for IABP in this setting is now strongly challenged.(19) In this study, we found that the order of IABP insertion and primary PCI was not associated with 30-day and late mortality. Our results can be considered to be in line with the results from the IABP-SHOCK II trial. The impact of IABP counterpulsation on infarct size has not been reported in the IABP-SHOCK II trial.

Some limitations of our study need to be acknowledged. Firstly, this study is not a randomized clinical trial but an observational retrospective cohort study. Despite using multivariable analysis to adjust for possible confounders that may be correlated to study outcomes, we cannot exclude the possibility of residual confounding. However, there were no significant differences in baseline characteristics between the two groups so that the outcomes could be more easily compared. Secondly, the golden standard for infarct size measurement is MRI. Because MRI was not routinely applied in our study population, serum peak CK concentration was used instead since it has been shown to have a good correlation with infarct size. Finally, this study may have lacked power to detect statistically significant differences in late mortality as well as infarct related pathologic Q-wave formation and cumulative residual ST-deviation in post-procedural ECGs.

In conclusion, the order of IABP insertion and primary PCI in STEMI patients presenting with cardiogenic shock was not associated with 30-day and late mortality. However, the results of this study suggest that early IABP insertion before primary PCI might be associated with higher peak CK levels, indicating a larger infarct size, when compared to IABP insertion directly after PCI. A possible explanation may be the increased reperfusion delay due to the time required for insertion of the IABP. As such, the results of the present study suggest that achievement of early reperfusion with primary PCI could have priority over routine early IABP insertion in STEMI patients with cardiogenic shock. Randomized studies are needed to determine the optimal timing of IABP insertion relative to primary PCI in this high-risk patient population.

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Chapter 20

Usefulness of intra-aortic balloon pump counterpulsation in patients with cardiogenic shock from acute myocardial infarction

Cheng JM, Valk SDA, Den Uil CA, Van Der Ent M, Lagrand WK,
Van De Sande M, Van Domburg RT, Simoons ML.

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ABSTRACT

Although intra-aortic balloon pump (IABP) counterpulsation is increasingly being used for treatment of patients with cardiogenic shock from acute myocardial infarction (AMI), data on long-term outcome is lacking. The aim of this study was to evaluate 30-day and long-term mortality, and to identify predictors for 30-day and long-term all-cause mortality of patients with AMI complicated by cardiogenic shock who were treated with IABP. From January 1990 to June 2004, a cohort of 300 consecutive patients treated with IABP was included. Mean age of the study population was 61±11 years and 79% of the patients were men. Survival until IABP removal after successful hemodynamic stabilization was 70% (n=211). Overall cumulative 30-day survival was 58%. Thirty-day mortality decreased over time from 52% in 1990-1994 to 36% in 2000-2004 (p for trend <0.05). Follow-up ranged from 0 to 15 years. In patients who survived until IABP removal, cumulative 1-, 5-, and 10-year survival rates were 69%, 58% and 36%, respectively. Adjusted predictors of long-term mortality were arrhythmias during stay at intensive cardiac care unit (ICCU) (HR 1.8, 95% CI 1.2-2.9) and renal failure during ICCU stay (HR 2.5, 95% CI 1.3-5.1). After adjustment, treatment with primary percutaneous coronary intervention (PCI) (HR 0.5, 95% CI 0.3-0.9) and coronary artery bypass grafting (CABG) (HR 0.4, 95% CI 0.2-0.8) were associated with lower long-term mortality. In conclusion, in patients with AMI complicated by cardiogenic shock treated with IABP, 30-day survival improved over time, as also an encouraging number of patients survived at the long-term.

INTRODUCTION

Cardiogenic shock is a state of inadequate tissue perfusion due to cardiac dysfunction.[1] Incidence rate of cardiogenic shock complicating acute myocardial infarction (AMI) ranges from 5 to 10 percent.[2] Despite the fact that prognosis of patients with cardiogenic shock has improved over time due to aggressive reperfusion strategies, in-hospital mortality from cardiogenic shock remains very high (i.e. 50%).[2,3] Use of intra-aortic balloon pump (IABP) counterpulsation is associated with improved survival in cardiogenic shock patients treated with thrombolysis.[4-7] Although IABP use in cardiogenic shock adjunctive to primary percutaneous coronary intervention (PCI) was recently questioned,[8] IABP is the method of first choice for mechanical assistance in patients with cardiogenic shock who do not respond adequately to standard pharmacological treatment.[9] Also long-term follow-up data of these patients are lacking. Therefore, the aim of this study was to evaluate 30-day and long-term outcome, and to identify predictors of 30-day mortality of patients with cardiogenic shock treated with IABP. In addition, we evaluated predictors of long-term mortality in patients who survived until IABP removal after successful hemodynamic stabilization.

METHODS

Between January 1990 and June 2004, all consecutive patients (n=300) with cardiogenic shock from AMI who were treated with IABP at Erasmus Medical Center in Rotterdam, the Netherlands, were included. Our center is a tertiary referral center for PCI, mechanical treatment of cardiogenic shock and heart transplantation. Between 1990 and 2000, about 175 patients with AMI were admitted to our center annually. Between 2001 and 2004, the number of admitted AMI patients increased to 350 annually, since primary PCI was implemented as standard treatment of AMI and our center was referral center for primary PCI. The indication for IABP counterpulsation was cardiogenic shock in all cases. IABP insertion was withheld only when such was technically not feasible (e.g. because of severe atherosclerotic peripheral artery disease or aortic disease), as well as in patients judged to have a definite fatal prognosis because of concomitant disease, which concerned 21 patients during inclusion period. Data were acquired retrospectively from patient medical records and from the hospital database, where a missing rate of <5% was considered acceptable. We performed a sub-analysis in patients who survived until IABP removal after successful hemodynamic stabilization.

The IABP was inserted either at the catheterization laboratory or at the Intensive Cardiac Care Unit (ICCU). Between 1990 and 1995, Datascope (Datascope Corp., Fairfield, NJ, USA) 9.5 and 10.5 French catheters were used. From 1995 through 2000, Arrow (Arrow

Corp., Reading, PA, USA) 9 French catheters were used, and between 2000 and 2004, Arrow (Arrow Corp., Reading, PA, USA) 8 French catheters were used.

Recent acute myocardial infarction (AMI) was defined as acute myocardial infarction at time of hospital admission. Besides clinical evidence of AMI (i.e. typical chest pain and electrocardiographic abnormalities) all patients had a diagnostic rise in cardiac markers during the hospitalization period. Cardiogenic shock was defined as low systolic blood pressure (<90 mm Hg) due to cardiac insufficiency with clinical signs of hypoperfusion (cold extremities, oliguria, altered mental state etc.), not responsive to fluid resuscitation. Most patients already received inotropic agents prior to collection of baseline data. Blood pressure was measured just before IABP insertion (baseline). Left ventricular function (LVF) was assessed with echocardiography by trained cardiologists and categorized into normal (ejection fraction (EF) >40%) or impaired (EF <40%). Inotropic agents used in our hospital were catecholamines (dobutamine, dopamine, and/or norepinephrine) and phosphodiesterase inhibitors (enoximone). Anti-arrhythmic agents used in our hospital were lidocaine, amiodarone, sotalol and digoxin. The following complications of IABP counterpulsation were registered: limb ischemia requiring IABP removal, bleeding, embolic and thrombotic events, need for vascular surgery and IABP-related infection. Limb ischemia was defined as diminished or absent peripheral pulsation with a white coloration of the leg at the side of IABP catheter introduction. Major bleeding was defined as a bleeding requiring red blood cell transfusion. Minor bleeding was defined as any access site bleeding not requiring red cell transfusion. Infection was defined as fever, combined with leukocytosis, increased C-reactive protein level (> 5 mg/l), and signs of inflammation at the IABP insertion site, with or without positive blood and/or IABP-tip cultures.

Follow-up started at the day of IABP insertion (baseline). In March 2006, vital status of all patients was acquired from municipal civil registries with a response rate of 100%. Ten patients (3%) were lost during follow-up. Median follow-up duration was 6.1 years and ranged from 0 to 15 years.

All data were analyzed with SPSS software (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Continuous variables were compared by Student *t* test or one-way ANOVA test and are presented as mean ± standard deviation. Nonparametric continuous variables were compared by Mann-Whitney U test or Kruskal-Wallis test and are presented as median and range. Categorical variables were compared by chi-square test or Fisher's exact test, when appropriate, and are presented in percentages. Patients lost to follow-up were considered at risk until the date of last contact, at which timepoint they were censored. Cumulative survival was estimated according to the Kaplan-Meier method. Kaplan-Meier survival curves were compared by log-rank test. Univariate and multivariate logistic regression analyses were performed to identify predictors of 30-day all-cause mortality. Multivariate Cox proportional hazards regression analyses were performed to identify

predictors of long-term all-cause mortality in patients who survived until IABP removal. In multivariate analyses, the variables age, sex, diabetes mellitus, hypercholesterolemia, history, LVF, blood pressure, heart rate, IABP running time, ST-elevation myocardial infarction, reperfusion therapy and complications during ICCU stay were entered into the model in a stepwise fashion. The final results are presented as unadjusted and adjusted odds ratios (OR), and respectively as unadjusted and adjusted hazard ratios (HR), both with the associated 95% confidence intervals (95% CI). Subanalyses were performed stratified to IABP running time (1 day, 2-5 days, ≥6 days). All statistical tests were two-tailed, where a p-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics are presented in Table 1. Mean age of the study population was 61±12 years and 79% of the patients were male. Most patients had an ST-segment elevation myocardial infarction (92%). Reperfusion therapy was performed in 80% of the patients; 45% were treated with primary PCI, 27% were treated with thrombolysis, and 12% were treated with emergency coronary artery bypass grafting (CABG). Most patients already received inotropic agents prior to IABP insertion (82%). Mechanical ventilation and cardiopulmonary resuscitation during ICCU stay were needed in 56% and 33% respectively. Arrhythmias requiring use of anti-arrhythmic agents and renal failure requiring renal replacement therapy occurred in 44% and 6% of patients, respectively.

Baseline characteristics of patients who survived until IABP removal after successful hemodynamic stabilization (n=211) are presented in Table 2. IABP running time in this subgroup was 1 day in 18%, 2-5 days in 58%, and ≥6 days in 24% with a median of 3 days (range 1 to 27 days). History of prior CABG ($p<0.05$), impaired LVF ($p<0.01$), and arrhythmias ($p<0.001$) were more frequent in patients with IABP running time ≥6 days as compared to patients with an IABP running time of 2-5 days. Less patients with IABP running time ≥6 days were treated with primary PCI ($p<0.05$).

Cumulative survival untill IABP removal after successful hemodynamic stabilization was 70% (n=211) and overall cumulative 30-day survival was 58%. Patients who had an IABP for only one day had higher 30-day mortality compared to patients with an IABP running time of longer than one day. Mortality was highest in the first days after IABP insertion. Cumulative long-term survival was 48%, 41% and 25% at respectively 1, 5 and 10 years of follow-up. Ten year estimated cumulative survival in 30-day survivors was 41%. Cumulative long-term Kaplan-Meier survival estimates of patients who survived until IABP removal after successful hemodynamic stabilization are shown in Figure 1. In these patients, cumulative long-term survival was 69%, 58% and 36% at 1, 5 and 10 years of follow-up, respectively. Patients with an IABP running time of ≥6 days had significant

Table 1. **Baseline and procedural characteristics**	
	Total (n=300)
Age (years)	61±11
Gender (male)	79%
Risk factors	
Diabetes Mellitus	20%
Hypertension	35%
Smoking	55%
Hypercholesterolemia	27%
Peripheral vessel disease	9%
Renal insufficiency	3%
History	
Prior cerebral vascular accident	6%
Prior myocardial infarction	45%
Prior coronary artery bypass grafting	9%
Prior percutaneous coronary intervention	7%
Systolic blood pressure[a] (mm Hg)	102±31
Diastolic blood pressure[a] (mm Hg)	63±19
Heart rate (bpm)	99±26
Impaired left ventricular function	76%
Three vessel / left main stem coronary disease	42%
ST-segment elevation and location	
Anterior/septal ST-elevation myocardial infarction	57%
Inferior/posterior ST-elevation myocardial infarction	35%
Non-ST-elevation myocardial infarction	8%
Mechanical complication after myocardial infarction	12%
Reperfusion therapy	
Primary percutaneous coronary intervention	45%
Thrombolysis	27%
Coronary artery bypass grafting	12%
No reperfusion therapy	20%
Complications during stay at intensive cardiac care unit	
Cardiopulmonary resuscitation	33%
Mechanical ventilation	56%
Arrhythmias requiring anti-arrhythmic agents	44%
Renal failure requiring renal replacement therapy	6%
Intra-aortic balloon pump running time	
1 day	31%
2 to 5 days	49%
6 days or longer	20%

Values expressed as mean ± SD or percent.

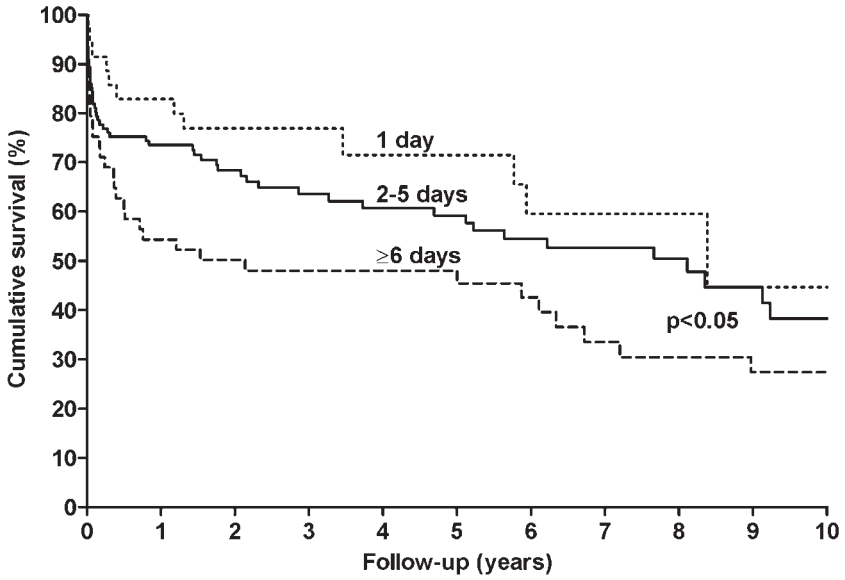
[a] Blood pressure measured just before insertion of intra-aortic balloon pump.

Table 2. Baseline and procedural characteristics of patients who survived until removal of the intra-aortic balloon pump after successful hemodynamic stabilization

	IABP running time				
	Total	1 day	2-5 days	≥6 days	
	(n=211)	(n=37)	(n=123)	(n=51)	P
Age (years)	60±11	61±12	60±11	59±12	0.7
Gender (male)	81%	84%	81%	80%	0.9
Risk factors					
Diabetes Mellitus	20%	16%	21%	22%	0.8
Hypertension	35%	44%	33%	34%	0.5
Smoking	56%	53%	56%	59%	0.9
Hypercholesterolemia	28%	35%	25%	31%	0.4
Peripheral vessel disease	8%	9%	8%	7%	0.9
Renal insufficiency	4%	3%	2%	8%	0.1
History					
Prior cerebral vascular accident	6%	5%	7%	4%	0.8
Prior myocardial infarction	43%	41%	38%	55%	0.1
Prior coronary artery bypass grafting	10%	16%	5%	18%	<0.05
Prior percutaneous coronary intervention	8%	14%	7%	6%	0.4
Systolic blood pressure[a] (mm Hg)	105±30	114±28	104±32	100±25	0.1
Diastolic blood pressure[a] (mm Hg)	64±17	69±16	63±18	62±16	0.2
Heart rate (bpm)	96±27	95±28	94±28	101±22	0.3
Impaired left ventricular function	73%	50%	74%	85%	<0.01
Three vessel / left main stem coronary disease	38%	41%	38%	35%	0.9
ST segment elevation and location					0.2
Anterior/septal ST-elevation myocardial infarction	57%	43%	59%	63%	
Inferior/posterior ST-elevation myocardial infarction	36%	46%	37%	27%	
Non-ST-elevation myocardial infarction	7%	11%	4%	10%	
Mechanical complication after myocardial infarction	11%	11%	13%	8%	0.7
Reperfusion therapy					
Primary percutaneous coronary intervention	47%	63%	49%	31%	<0.05
Thrombolysis	30%	23%	30%	37%	0.4
Coronary artery bypass grafting	15%	11%	14%	20%	0.4
No reperfusion therapy	13%	6%	11%	22%	0.1
Complications during stay at intensive cardiac care unit					
Cardiopulmonary resuscitation	26%	24%	24%	29%	0.8
Mechanical ventilation	51%	38%	51%	59%	0.1
Arrhythmias requiring anti-arrhythmic agents	48%	19%	46%	73%	<0.001
Renal failure requiring renal replacement therapy	6%	0%	6%	12%	0.1

Values expressed as mean ± SD or percent.

[a] Blood pressure measured just before insertion of intra-aortic balloon pump.



1 day						
Number died	0	0	11	13	13	14
Number at risk	37	24	14	9	7	2
2-5 days						
Number died	0	38	44	48	50	54
Number at risk	123	62	43	32	21	8
≥6 days						
Number died	0	26	27	29	33	34
Number at risk	51	24	22	16	11	9

Figure 1. Cumulative long-term survival in patients with cardiogenic shock who survived until removal of the intra-aortic balloon pump after successful hemodynamic stabilization, stratified by intra-aortic balloon pump running time.
P-value is obtained by comparing intra-aortic balloon pump running time of ≥6 days with intra-aortic balloon pump running time of 2-5 days.

higher long-term mortality compared to patients with an IABP running time of 2 to 5 days ($p<0.05$).

Unadjusted predictors of 30-day and long-term mortality are presented in Table 3. Adjusted predictors of 30-day mortality are presented in Figure 2. In multivariate analysis, adjusted predictors of 30-day mortality were age (OR 1.04, 95% CI 1.01-1.07), impaired LVF (OR 2.5, 95% CI 1.1-5.5), need for cardiopulmonary resuscitation (OR 2.6, 95% CI 1.4-4.9), and need for mechanical ventilation (OR 2.4, 95% CI 1.3-4.5). After adjustment, treatment with primary PCI (OR 0.1, 95% CI 0.1-0.3), thrombolysis (OR 0.1, 95% CI 0.0-0.4), and emergency CABG (OR 0.1, 95% CI 0.0-0.4) were associated with improved 30-day survival. Adjusted predictors of long-term mortality in patients who survived until IABP removal after successful hemodynamic stabilization are presented in Figure 3. Adjusted predictors of long-term mortality were arrhythmias requiring use of anti-arrhythmic agents (HR 1.8, 95% CI 1.2-2.9) and renal failure requiring renal replacement therapy

Table 3. Unadjusted predictors of 30-day and long-term mortality

	30-day mortality		Long-term mortality[a]	
	OR	95% CI	HR	95% CI
Age	1.03	1.01-1.05	1.02	1.00-1.04
Male	0.7	0.4-1.2	1.1	0.7-1.7
Risk factors				
Diabetes Mellitus	1.1	0.6-1.9	1.2	0.8-1.9
Hypertension	1.0	0.6-1.8	1.1	0.7-1.7
Smoking	0.7	0.4-1.1	0.7	0.5-1.1
Hypercholesterolemia	0.7	0.4-1.2	0.9	0.6-1.4
History				
Prior cerebral vascular accident	1.3	0.5-3.4	1.8	0.9-3.5
Prior myocardial infarction	1.5	0.9-2.4	1.5	1.0-2.2
Prior coronary artery bypass grafting	0.8	0.4-1.8	1.8	1.0-3.1
Prior percutaneous coronary intervention	0.5	0.2-1.4	1.1	0.5-2.3
Impaired left ventricular function	2.8	1.5-5.2	1.8	1.2-2.7
Systolic blood pressure, units of 10 mmHg	0.9	0.8-1.0	1.0	1.0-1.1
Diastolic blood pressure, units of 10 mmHg	0.9	0.8-1.0	1.0	0.9-1.1
Heart rate, units of 10 bpm	1.1	1.0-1.2	1.0	1.0-1.1
Hospital admission in 1995-1999[b]	0.6	0.3-1.2	0.7	0.4-1.1
Hospital admission in 2000-2004[b]	0.5	0.3-0.9	0.7	0.4-1.1
IABP running time of 1 day[c]			0.8	0.5-1.5
IABP running time of ≥6 days[c]			1.6	1.0-2.4
ST-elevated myocardial infarction	2.7	1.6-2.3	1.3	0.6-2.9
Reperfusion therapy				
Primary percutaneous coronary intervention	0.4	0.2-0.7	0.7	0.4-1.2
Thrombolysis	0.2	0.1-1.0	0.7	0.3-2.2
Coronary artery bypass grafting	0.2	0.1-0.5	0.5	0.2-0.9
Cardiopulmonary resuscitation	2.5	1.5-4.1	1.2	0.8-1.8
Mechanical ventilation	2.0	1.3-3.2	1.4	0.9-2.0
Arrhythmias requiring anti-arrhythmic agents	0.9	0.6-1.4	1.7	1.2-2.6
Renal failure requiring renal replacement therapy	1.3	0.5-3.4	2.7	1.5-5.1

[a] Only patients who survived until removal of the intra-aortic balloon pump after successful hemodynamic stabilization were included in long-term cox regression analysis.
[b] Relative to hospital admission in 1990-1994.
[c] Relative to IABP running time of 2-5 days.

during ICCU stay (HR 1.6, 95% CI 1.1-2.4). Treatment with primary PCI (HR 0.5, 95% CI 0.3-0.9) and CABG (HR 0.4, 95% CI 0.2-0.8) were associated with improved survival at the long term.

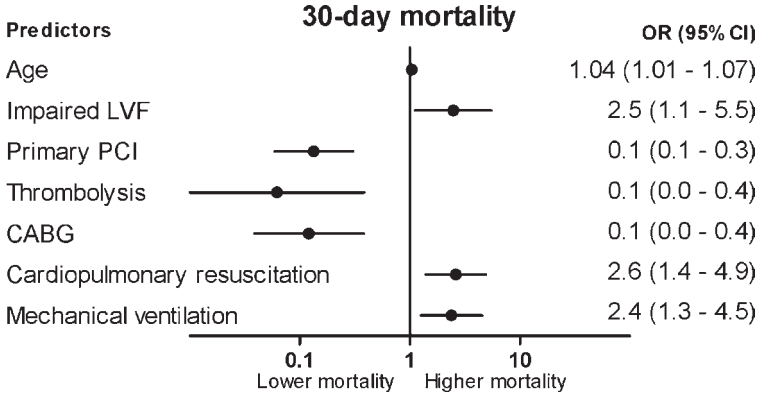


Figure 2. Adjusted predictors of 30-day mortality in patients with cardiogenic shock treated with an intra-aortic balloon pump, presented as odds ratios and as hazard ratios respectively, both with the associated 95% confidence intervals.

CABG = coronary artery bypass grafting; LVF = left ventricular function; PCI = percutaneous coronary intervention.

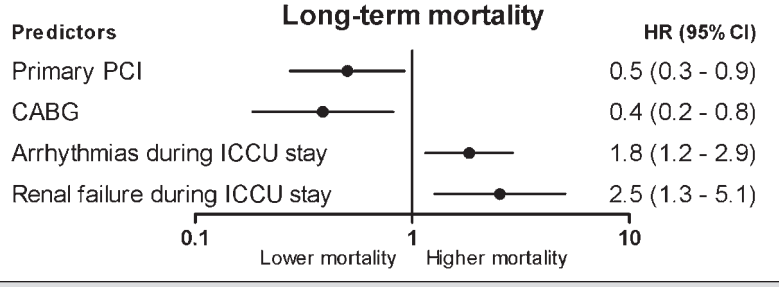


Figure 3. Adjusted predictors of long-term mortality in patients with cardiogenic shock treated with an intra-aortic balloon pump, presented as odds ratios and as hazard ratios respectively, both with the associated 95% confidence intervals.

Only patients who could be successfully weaned from IABP were included in long-term Cox regression analysis. Variables age, sex, diabetes mellitus, hypercholesterolemia, history, LVF, blood pressure, heart rate, IABP running time, period of hospital admission, ST-elevation myocardial infarction, reperfusion therapy and complications during ICCU stay were entered into the model in a stepwise fashion.

CABG = coronary artery bypass grafting; ICCU = intensive cardiac care unit; PCI = percutaneous coronary intervention.

Temporal trends of IABP use between 1990 to 2004 are shown in Table 4. Frequency of IABP insertion increased over the years from 69 patients with cardiogenic shock in 1990-1994 to 132 patients in 2000-2004. Frequency of primary PCI increased from 22% in 1990-1994 to 65% in 2000-2004 ($p<0.001$), while thrombolytic therapy was less frequently administered over time ($p<0.01$). Thirty-day mortality decreased from 52% in 1990-1994 to 36% in 2000-2004 (p for trend <0.05).

Table 4. Temporal trends of intra-aortic balloon pump use between 1990 to 2004

	1990-1994 (n=69)	1995-1999 (n=99)	2000-2004 (n=132)	P
Age (years)	61±10	58±12	63±11	<0.01
Gender (male)	81%	75%	81%	0.8
Reperfusion therapy				
Primary percutaneous coronary intervention	22%	34%	65%	<0.001
Thrombolysis	31%	37%	18%	<0.01
Coronary artery bypass grafting	19%	13%	7%	<0.05
No reperfusion therapy	34%	23%	11%	<0.001
Median intra aortic balloon pump running time (days)	3	3	2	<0.01
Any intra aortic balloon pump related complication	32%	26%	10%	<0.001
30-day mortality	52%	41%	36%	<0.05

Values expressed as mean ± SD, median or percent.

The overall incidence of IABP-related complications was 20%. Infection (9%), bleeding (6%), and limb ischemia (5%) were the most frequently observed complications. Limb ischemia was mostly transient with either spontaneous recovery or recovery after IABP removal. However, vascular surgery was needed in 2 patients. Five patients had a major bleeding at the access site requiring blood transfusion. Balloon rupture of the IABP occurred in 5 patients. The number of complications decreased from 32% in 1990-1994 to 10% in 2000-2004 (p<0.001).

DISCUSSION

To our knowledge, this is the first study presenting long-term follow-up of a large cohort of patients with cardiogenic shock from AMI all treated with IABP counterpulsation. Despite high 30-day mortality, a considerable amount of patients (36%) survived until 10 years after successful weaning of the intra-aortic balloon pump.

Thirty-day mortality in our study (42%) was comparable to mortality rates reported from other studies on cardiogenic shock patients. [2,4,10,11]. GUSTO-I investigators presented long-term outcome of a general group of AMI patients, of which some had cardiogenic shock.[12] This study showed a 10-year survival rate of 54% in 30-day survivors of cardiogenic shock. In the present study, we found a lower 10-year survival rate (41%) in these patients. However, we have to consider that not all cardiogenic shock patients in the GUSTO-I study were treated with IABP. Therefore, our study population might have been a higher risk population compared to GUSTO-I patients.

Thirty-day mortality was highest in patients who had an IABP inserted for less than one day. This group mainly consisted of severely compromised patients who could not

be stabilized in time despite hemodynamic support from the IABP and other treatment modalities, and who did not survive the first day after IABP insertion. Mortality with IABP in place in this group was 61% on the first day. In contrast, the remaining patients (39%) were in a better hemodynamic condition compared to patients with IABP running time >1 day, which explains their better 30-day outcome. Conversely, in patients who survived until IABP removal, patients who were treated with IABP for ≥6 days had higher long-term mortality compared to patients with IABP running time of 2-5 days. However, IABP running time was not an independent predictor of long-term mortality.

Adjusted predictors of 30-day mortality were age, impaired LVF, need for cardiopulmonary resuscitation and need for mechanical ventilation. Various reperfusion therapies (i.e., PCI, thrombolysis and CABG) were associated with lower 30-day mortality after adjustment. US National Registry of Myocardial Infarction 2 (NRMI2) investigators reported higher age, female gender, diabetes mellitus and a history of prior congestive heart failure as independent predictors of in-hospital mortality.[11] Predictors of long-term outcome of cardiogenic shock patients treated with IABP counterpulsation have not been presented previously in literature. For patients who could be successfully weaned from IABP, we report a strong correlation of arrhythmias and renal failure during ICCU stay on long-term mortality, while treatment with primary PCI and CABG was also beneficial at the long-term.

Although admission period was not an independent predictor of outcome, 30-day survival rate improved over time. This might be related to the implementation of early revascularization with primary PCI in the routine treatment of AMI complicated by cardiogenic shock.[13-15] Improving outcomes over time of cardiogenic shock patients were also found in a recent study.[2]

Incidence of major complications was in line with the incidence reported by the Benchmark registry.[16] In our study severe bleeding, major limb ischemia and balloon leak occurred in 2%, 1% and 2% respectively, compared to 0.8%, 0.9% and 1.0% respectively as reported by the Benchmark registry. The number of vascular complications decreased over the years, probably due to improved IABP-devices (especially the introduction of smaller sized catheters) as well as increased physician's skills and experience in time, which was also demonstrated in previous studies.[17-19] We reported an IABP-related incidence of infection of 9%. However, this relatively high rate of infection might be overestimated due to the robust definition of infection used in our study. Unfortunately, in most cases, IABP-tip and/or blood cultures to definitely prove IABP-related infections were not available in our study.

Other limitations of our study include the retrospective nature and the fact that we only included patients with cardiogenic shock who were treated with IABP. Thus, caution is urged in extrapolating these results to IABP-ineligible patients or to patients in cardiogenic shock who were not treated with IABP. Inclusion of a control group of

patients with cardiogenic shock without IABP treatment was not possible since IABP counterpulsation has been part of routine treatment of patients with AMI complicated by cardiogenic shock for years in our center. Hence, the benefit of IABP counterpulsation could not be proven by our study. Recently, a randomized controlled trial (www.clinicaltrials.gov: NCT00491036) has been initiated which will address the potential benefit of IABP counterpulsation adjunctive to medical treatment and primary PCI in patients with cardiogenic shock.

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Chapter 21

The intra-aortic balloon pump keeps pumping, but in selected patients

Den Uil CA, Cheng JM, Jewbali LSD, Nieman K, Akkerhuis KM, Dubois EA, Zijlstra F.

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ABSTRACT

In recent years, three randomised controlled trials questioned the use of the intra-aortic balloon pump (IABP) in populations of high-risk PCI (BCIS-1), acute myocardial infarction (CRISP-AMI), and cardiogenic shock (IABP-SHOCK II). This review describes these and other IABP trials, as well as their outcome on mortality. Clinical implications are addressed. There is a pressing need for adequately powered randomised controlled trials testing the optimal timing of mechanical circulatory support as well as device design in cardiogenic shock.

INTRODUCTION

Dr. Adrian Kantrowitz introduced the intra-aortic balloon pump (IABP) in the 1960s (*figure 1*). He used large balloon catheters inserted in the aorta by surgical cut down of the femoral artery. From the late 1970s placement of the balloon was modified using the Seldinger technique. Until recently, the intra-aortic balloon pump was increasingly being used to support haemodynamics in patients with acute myocardial infarction complicated by cardiogenic shock, ventricular septal rupture or acute mitral regurgitation,[1,2] as well as in patients undergoing high-risk percutaneous coronary intervention (PCI), prior to high-risk cardiac surgery,[3,4] in postcardiotomy cardiogenic shock, refractory heart failure,[5] and with refractory ventricular arrhythmias.[6] Its physiological effects in reducing afterload and improving coronary perfusion were believed to enhance survival in these groups of patients, especially because complication rates (access site bleeding and vascular complications) were low.[1,7] However, evidence supporting the use of IABP in these clinical settings was mainly based on registry data.[8] In recent years, three randomised controlled trials questioned the use of IABP in subsequently high-risk PCI, acute myocardial infarction, and cardiogenic shock. The aim of this review is to discuss these trials and to evaluate the current role of intra-aortic balloon pump counterpulsation in high-risk PCI and acute myocardial infarction with or without shock.

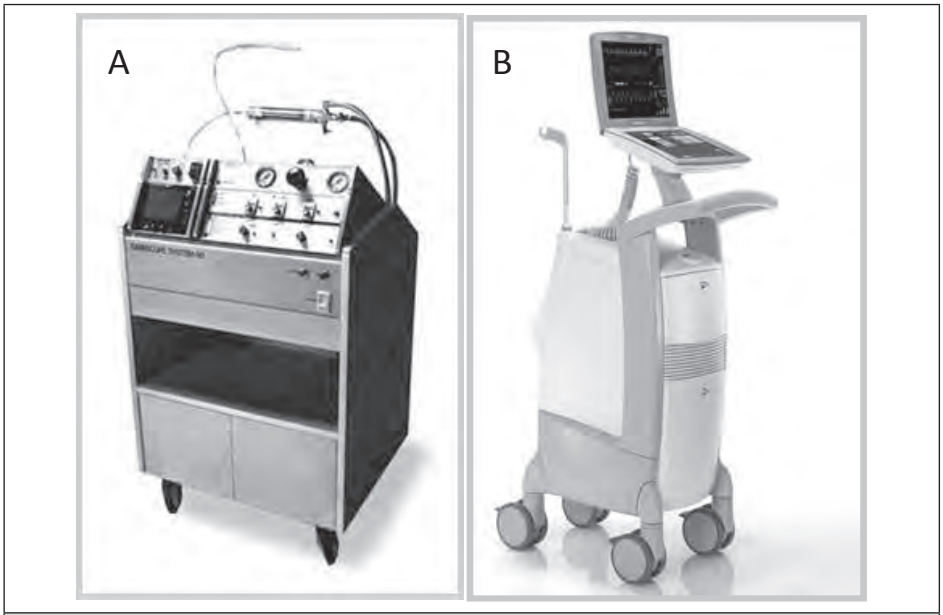

Figure 1. Development in balloon pump consoles.
Panel A: One of the first IABP consoles. Panel B: Modern miniaturized portable console. Courtesy of MAQUET Cardiovascular, Datascope Corp., Mahwah, NJ, USA.

HAEMODYNAMICS

The intended haemodynamic relief from the IABP is twofold. During diastole, the IABP inflates thereby displacing blood from the descending aorta. It then deflates immediately before systole creating a void in the aorta.[9] These mechanisms produce the haemodynamic effects of increasing diastolic, mean arterial and coronary perfusion pressures, while decreasing afterload. In serial measurements, IABP counterpulsation acutely lowers left ventricular end-diastolic pressure and modestly increases the cardiac index and cardiac power index.[10] Sustained haemodynamic benefit was contradicted by the results of a recent small randomised trial.[11] These investigators demonstrated that, in patients with complicated myocardial infarction, haemodynamic improvement in the days following IABP implantation did not significantly differ from the control group. However, this study was clearly underpowered and baseline haemodynamic parameters significantly differed between the two treatment arms.[11]

HIGH-RISK PERCUTANEOUS CORONARY INTERVENTION

The Balloon-Pump Assisted Coronary Intervention Study (BCIS-1) investigated balloon pump support in 301 elective patients undergoing high-risk PCI: left ventricular ejection fraction (LVEF) ≤ 30% and unprotected left main coronary artery or target vessel supply ≥40% of myocardium (*table 1*).[12] Prolonged procedural hypotension occurred more frequently in the group with no planned IABP insertion. Moreover, rescue IABP insertion was required in 18 patients (12%) assigned to have no planned IABP insertion. There was no difference in major adverse cardiac events and all-cause mortality at six-month follow-up. At long-term follow-up, elective IABP support during PCI was associated with a 34% relative reduction in all-cause mortality compared with unsupported PCI ($p = 0.04$, absolute difference 11%).[13]

ACUTE MYOCARDIAL INFARCTION

Several randomised trials investigated the use of IABP support in patients with large, mainly anterior wall, myocardial infarction without evidence of cardiogenic shock (*table 2*). Most studies demonstrated that systematic use of intra-aortic balloon pumping after primary angioplasty or coronary stenting did not lead to myocardial salvage, nor to a better clinical outcome in terms of major adverse cardiac events or all-cause mortality. However, most studies were underpowered to detect a difference in mortality. Counterpulsation Reduces Infarct Size pre-PCI (CRISP) was a large randomised trial (337 patients)

Table 1. Characteristics and outcome of randomized controlled clinical trials on intra-aortic balloon pump support compared to conventional therapy in high-risk elective PCI.

Trial (year)	Population	Intervention (I/C)	Sample size	Intervention not performed in I	Intervention performed in C	Median duration of support	Longest reported time point	Number of deaths (I/C)	OR or HR for mortality
BCIS-1 (2010)[12]	High-risk PCI	PCI+IABP/PCI	151/150	3(2%)	18(12%)	<24 h	6-month	7/151 11/150	0.61 [0.24-1.62]
BCIS-1 (2013)[13]	High-risk PCI	PCI+IABP/PCI	151/150	3(2%)	18(12%)	<24 h	51-month	42/151 58/150	0.66 [0.44-0.98]

I=intervention (IABP), C=control (medical management). PCI= percutaneous coronary intervention; IABP=intra-aortic balloon pump; OR=odds ratio; HR=hazard ratio.

Table 2. Characteristics and outcome of randomized controlled clinical trials on intra-aortic balloon pump support compared to conventional therapy in acute myocardial infarction without shock.

Trial (year)	Population	Intervention (I/C)	Sample size	Intervention not performed in I	Intervention performed in C	Median duration of support	Longest reported time point	Number of deaths (I/C)	OR or HR for mortality
Ohman (1994)[26]	Primary PTCA	PTCA+IABP/PTCA	96/86	0(0%)	7(8%)	48 h	In-hospital	2/96 2/86	0.90 [0.13-6.22]
PAMI-II (1997)[27]	Primary PTCA	PTCA+IABP/PTCA	211/226	29(14%)	26(12%)	36-48 h	In-hospital	9/211 7/226	1.38 [0.52-3.63]
Van 't Hof (1999)[28]	Primary or rescue PTCA	PTCA+IABP/PTCA	118/120	30(25%)	37(31%)	48 h	6-month	12/118 9/120	1.36 [0.59-3.10]
Vijayalakshmi (2007)[29]	High-risk PCI	PCI+IABP/PCI	17/16	Not reported	Not reported	48 h	In-hospital	3/17 0/16	6.61 [0.37-118.7]
CRISP AMI (2011)[14]	Primary PCI	PCI+IABP/PCI	161/176	8(5%)	15(9%)	<24 h	6-month	3/161 9/176	0.36 [0.10-1.32]
Gu (2011)[30]	Primary PCI	PCI+IABP/PCI	51/55	Not reported	Not reported	48 h	6-month	9/51 18/55	0.54 [0.27-1.09]

I=intervention (IABP), C=control (medical management). PTCA=percutaneous translumincal coronary angioplasty; PCI= percutaneous coronary intervention; IABP=intra-aortic balloon pump; OR=odds ratio; HR=hazard ratio.

that evaluated the role of IABP support started prior to PCI in anterior STEMI without cardiogenic shock.[14] No difference was observed in the primary endpoint (infarct size assessed by cardiac magnetic resonance imaging) in the IABP group, compared with the control group. All-cause mortality at six months was less frequent in the IABP group, but the number of events was low and not significantly different (1.9 vs. 5.2%, *table 2*). Preliminary observational data suggest that IABP insertion before primary PCI might in fact result in larger infarct sizes,[7] presumably due to the (short) delay in coronary revascularisation (myocardial reperfusion) associated with upstream implantation of the pump.

ACUTE MYOCARDIAL INFARCTION COMPLICATED BY CARDIOGENIC SHOCK

The IABP-SHOCK II trial randomised 600 patients with acute myocardial infarction complicated by cardiogenic shock to IABP support or not.[15] Ninety-six percent of the patients received primary PCI. The primary endpoint of 30-day all-cause mortality was not different between the two treatment arms (40% in the IABP group vs. 41% in controls, p = 0.69, *table 3*).

The sample size calculation for the IABP SHOCK II trial was based on an anticipated 30-day mortality of 56% in the control group, where the observed mortality rate was about 40%. Therefore, despite having randomised 600 patients, the trial still lacked sufficient power to address its primary hypothesis definitively.[16] In addition, several other issues thwart interpretation of the study results. First, there were no haemodynamic requirements to confirm the diagnosis of cardiogenic shock. Second, in one-fourth of the patients, the right coronary artery was the infarct-related vessel and right ventricular infarction (which is not a good indication for IABP) might have contributed to haemodynamic compromise. Third, since a study using balloon pumps (in sick patients) could not be blinded, cross-over occurred in 10% of the control arm. Fourth, ten patients assigned to the IABP group died before an IABP could be inserted, which might have influenced the results. Finally, all-cause, and not cardiovascular, mortality rates were reported. Since nearly half of the patients received cardiopulmonary resuscitation before randomisation, it is possible that a neurological cause of death (which is not positively affected by IABP) may have superseded cardiovascular death.

Table 3. Characteristics and outcome of randomized controlled clinical trials on intra-aortic balloon pump support compared to conventional therapy in acute myocardial infarction complicated by cardiogenic shock.

Trial (year)	Population	Intervention (I/C)	Sample size	Intervention not performed in I	Intervention performed in C	Median duration of support	Longest reported time point	Number of deaths (I/C)	OR or HR for mortality
TACTICS (2005)[31]	Thrombolysis or rescue PCI	TT±PCI+IABP/ TT±PCI	30/27	3(10%)	9(33%)	48 h	6-month	10/30 12/27	0.75 [0.39-1.45]
IABP-SHOCK II (2012)[15]	Primary PCI	PCI+IABP/ PCI	300/298	13(4%)	30(10%)	72 h	30-days	119/300 123/298	0.96 [0.79-1.17]
IABP-SHOCK II (2013)[25]	Primary PCI	PCI+IABP/ PCI	299/296	13(4%)	30(10%)	72 h	12-month	155/299 152/296	1.01 [0.86-1.18]

I=intervention (IABP), C=control (medical management). PCI= percutaneous coronary intervention; IABP=intra-aortic balloon pump; OR=odds ratio; HR=hazard ratio.

Table 4. Characteristics and outcome of randomized controlled clinical trials on intra-aortic balloon pump compared to percutaneous left ventricular assist device (LVAD) support.

Trial (year)	Population	Intervention (I/C)	Sample size	Intervention not performed in I	Intervention performed in C	Median duration of support	AMI	Shock	Longest reported time point	Number of deaths (I/C)	OR or HR for mortality
PROTECT-II (2012)[18]	High-risk PCI	PCI+Impella 2.5/ PCI+IABP	225/222	0 (0%)	0 (0%)	LVAD 1.9 h/ IABP 8.4 h	No	No	3 month	19/222 27/225	0.71 [0.41-1.24]
Thiele (2005)[19]	Cardiogenic shock	PCI+TandemHeart/ PCI + IABP	21/20	0(0%)	1(5%)	LVAD 96 h/ IABP84 h	Yes	Yes	30-day	9/20 9/21	0.95 [0.48-1.90]
Burkhoff (2006)[20]	Cardiogenic shock	TandemHeart/ IABP	19/14	0(0%)	0(0%)	LVAD 48 h/ IABP 46 h	70%	Yes	30-day	9/19 5/14	1.33 [0.57-3.10]
Seyfarth (2008)[21]	Cardiogenic shock	Impella 2.5/ IABP	13/13	1(8%)	1(8%)	LVAD 25 h/ IABP 23 h	Yes	Yes	30-day	6/13 6/13	1.00 [0.44-2.29]

I=intervention (percutaneous LVAD), C=control (IABP). PCI= percutaneous coronary intervention; IABP=intra-aortic balloon pump; OR=odds ratio; HR=hazard ratio.

IABP VS. PERCUTANEOUS LEFT VENTRICULAR ASSIST DEVICES

The more powerful percutaneous left ventricular assist devices (pLVADs) can directly unload the left ventricle by withdrawing blood from the left atrium after transseptal puncture (TandemHeart) or, somewhat less invasively, by aspirating blood from the left ventricle through retrograde crossing of the aortic valve (Impella).[17] pLVADS would potentially afford the left ventricle with more haemodynamic support. The PROTECT-II study randomised patients undergoing elective high-risk PCI (last patent vessel with LVEF ≤35%, unprotected left main, or three-vessel disease with LVEF ≤30%) to support with either IABP or Impella 2.5.[18] The primary endpoint (a composite of 30-day major adverse events) was not statistically different between the two groups. However, there was a trend for less major adverse events associated with Impella support at 90 days. All-cause mortality was not different at one- and three-month follow-up (*table 4*).

Three small randomised trials compared TandemHeart[19,20] or Impella[21] versus IABP support in the setting of cardiogenic shock. Use of these percutaneous LVAD systems provided superior haemodynamic support compared with the IABP; however, this did not translate into lower 30-day mortality (pooled relative risk 1.06 [0.68-1.66]).[22] Significant bleeding was observed more frequently in patients treated with a TandemHeart.

CONCLUSIONS AND CURRENT PERSPECTIVES

Most of the above-mentioned IABP trials could not demonstrate a clinical benefit; however, almost all these trials, especially when mortality was used as endpoint, were underpowered. When we take this into account, which conclusions could be drawn from these trials? First, based on BCIS-1, IABP support may lower mortality in the setting of high-risk elective PCI, where the Impella device remains an alternative, more powerful, way of support, especially if a higher output can be generated such as may be accomplished with the Impella 5.0.[13,18,23] Second, CRISP-AMI demonstrated that routine implantation of an IABP in acute myocardial infarction is not useful and this result is in line with other observations.[14] Finally, the IABP-SHOCK II trial data are difficult to interpret. We may conclude that routine IABP implantation beyond common intropic support in cardiogenic shock probably has no benefit.[24,25] The problem in these 'crash and burn' patients, however, is that there is no proven alternative for the balloon pump in case of inevitably necessary mechanical circulatory support. We are still awaiting the results of an adequately powered randomised trial of a percutaneous left ventricular assist device (probably Impella) in cardiogenic shock, compared with IABP. In the meantime the IABP keeps pumping, while we propose restricted use in carefully selected patients undergoing high-risk PCI or those with evidence of persistent circulatory failure despite pharmacological support.

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EPILOGUE

Summary and conclusions

Nederlandse samenvatting

Dankwoord / Acknowledgements

Curriculum vitae

List of scientific publications

List of scientific presentations

PhD portfolio

SUMMARY AND CONCLUSIONS

Chapter 1 describes the rationale and study design of the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study. This large, prospective, observational cohort study aims to investigate the relation of novel circulating biomarkers with coronary plaque characteristics as determined by intravascular ultrasound virtual histology (IVUS-VH) and near-infrared spectroscopy (NIRS). Furthermore, this study aims to investigate the prognostic value of novel biomarkers and plaque phenotypes as determined by IVUS and NIRS for occurrence of major adverse cardiac events.

Chapter 2 describes the prognostic value of in-vivo detection of high risk plaques by IVUS-VH in patients with established coronary artery disease. The presence of thin-cap fibroatheroma lesions (which are believed to be the most prevalent substrate of atherosclerotic plaque rupture resulting in acute coronary syndrome) on IVUS-VH is strongly and independently predictive for occurrence of death and acute coronary syndrome. Thin-cap fibroatheroma lesions with a large plaque burden carry higher risk than smaller thin-cap fibroatheroma lesions, especially on the short term.

Chapter 3 describes the prognostic value of intracoronary NIRS. The lipid core burden index (LCBI) as assessed by NIRS in a non-culprit coronary artery is associated with major adverse cardiac events (MACE) during 1-year of follow-up in patients with established coronary artery disease who were referred for coronary angiography. Patients with an LCBI above the median of 43.0 had a 4-fold risk. This observation warrants confirmation by larger studies with extended follow-up.

Chapter 4 describes the association between high-sensitivity troponin-T and coronary plaque characteristics assessed with IVUS-VH in patients with stable coronary artery disease. Coronary plaque volume and the presence of IVUS-VH-derived thin-cap fibroatheroma lesions are associated with higher circulating troponin-T levels. 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.

Chapter 5 describes the association between high-sensitivity C-reactive protein (CRP) and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. CRP is a marker of coronary plaque burden, but is not related to the presence of IVUS-VH-derived thin-cap

fibroatheroma lesions and stenotic lesions. CRP levels >3 mg/L are predictive for adverse cardiovascular outcome at 1 year.

Chapter 6 describes the association between circulating cytokines and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Higher circulating tumor necrosis factor-α (TNF-α) was associated with with higher plaque burden and IVUS-VH-derived thin-cap fibroatheroma lesions in patients with stable coronary artery disease. Lower interleukin-10 was associated with higher plaque burden and large IVUS-VH-derived thin-cap fibroatheroma lesions. These in-vivo findings suggest a role for these cytokines in extent and vulnerability of atherosclerosis.

Chapter 7 describes the association between circulating acute phase proteins and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Higher circulating ferritin was associated with higher coronary plaque volume, and higher PAI-1 was associated with higher incidence of all-cause mortality or acute coronary syndrome. None of the acute phase proteins displayed consistent associations with composition of atherosclerosis or plaque vulnerability.

Chapter 8 describes the association between circulating chemokines and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Regulation upon activation normal T-cell expressed and secreted (RANTES) is a promising biomarker that is inversely associated with coronary plaque burden and vulnerability, as well as with death and acute coronary syndrome.

Chapter 9 describes the association between antibodies against periodontal pathogens and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Plasma IgG and IgA against major periodontal pathogens were not associated with the extent of coronary atherosclerosis (with exception of a trend in diabetics) nor with coronary plaque vulnerability. IgA against periodontal pathogens were inversely associated with extent of coronary remodeling. Altogether, these results do not add evidence for a substantial role of systematic exposure to periodontal pathogens in coronary artery disease.

Chapter 10 describes the association between proprotein convertase substilisin/kexin type 9 (PCSK9) and coronary plaque inflammation assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Serum

PCSK9 level is linearly associated with the fraction and amount of necrotic core tissue in coronary atherosclerosis and with 1-year clinical outcome after coronary angiography, independently of serum low-density lipoprotein (LDL) level and statin use. PCSK 9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL regulation.

Chapter 11 describes the association of eight previously identified high risk cholesteryl ester, ceramide and lactosylceramide lipids and three ceramide ratios with coronary plaque characteristics assessed with IVUS-VH and NIRS imaging, as well as with 1-year clinical outcome in a large cohort of patients with established coronary artery disease. The main finding is that higher plasma concentrations of several of these molecular lipid species are associated with more vulnerable plaque morphology, reflected by a higher fraction of coronary plaque consisting of necrotic core tissue on IVUS-VH and by a higher LCBI on NIRS imaging. Additionally, Cer(d18:1/16:0) and the ceramide ratios predict 1-year cardiovascular outcome.

Chapter 12 describes the association between von Willebrand factor (VWF) and coronary plaque characteristics assessed with IVUS-VH in patients with established coronary artery disease who underwent coronary angiography. Higher coronary plaque burden was associated with higher circulating VWF levels in patients with stable coronary artery disease. High VWF levels are predictive of adverse cardiovascular outcome and death during one-year follow-up

Chapter 13 describes the prognostic value of high-sensitivity CRP during 10-year follow-up after percutaneous coronary intervention (PCI). Higher CRP levels at the time of the procedure are predictive for 10-year mortality and myocardial infarction. Although adding CRP to a prediction model containing conventional cardiovascular risk factors did not significantly improve discriminatory power, CRP was able to improve risk classification. Therefore, CRP might be an useful biomarker to further improve risk assessment in patients undergoing PCI.

Chapter 14 describes the associations between thirteen circulating plasma biomarkers that were previously identified by proteomics or immunohistochemistry experiments in human carotid plaques, and adverse cardiovascular outcome in patients undergoing coronary angiography. OGN and NGAL/MMP9 are promising biomarkers that are expressed in vulnerable atherosclerotic plaques and that are independently predictive for occurrence of major adverse cardiovascular events within 1 year after coronary angiography. Both biomarkers displayed incremental value over conventional cardiovascular risk factors and CRP in terms of risk reclassification.

Chapter 15 describes the associations between plasma levels of 42 cytokines, chemokines and growth factors measured with a multiplex assay and adverse cardiovascular outcome in patients with established coronary artery disease. We found that 3 out of the 42 biomarkers, specifically IL18, CXCL8 and CXCL9, could be potential predictors of cardiovascular outcome in patients with established coronary artery disease.

Chapter 16 describes the development of simple risk chart for early assessment of 30-day mortality risk in patients with cardiogenic shock from ST-elevation myocardial infarction (STEMI) admitted to undergo primary PCI. Overall 30-day mortality was 38.4% and did not change over the years between 2000 and 2012. Mortality can be well predicted already at time of hospital admission by a risk chart that uses only three variables, namely age, initial serum lactate, and creatinine level. The risk chart was able to stratify patient's mortality risk into categories ranging from 0-20% to 80-100%, and had a better performance than the GRACE risk score.

Chapter 17 describes the prognostic value of sublingual perfused capillary density using sidestream dark field imaging as a measure of tissue perfusion in patients with cardiogenic shock from acute myocardial infarction. Diminished sublingual perfused capillary density, at baseline or following treatment, is associated with the development of multi-organ failure and is a predictor of 30-day mortality. Assessment of sublingual perfused capillary density may be considered as a simple non-invasive tool to assess shock severity and mortality risk in patients with cardiogenic shock.

Chapter 18 is a meta-analysis of controlled trials that compared percutaneous left ventricular assist devices (LVADs) with intra-aortic balloon pump (IABP) counterpulsation in patients with cardiogenic shock. The use of a percutaneous LVAD provides superior hemodynamic support compared to the use of IABP. However, this hemodynamic benefit of the percutaneous LVAD did not result into a reduced 30-day mortality rate. Furthermore, a higher rate of adverse events was encountered by the higher invasive nature of LVAD, especially of the TandemHeart device. Until now, we cannot recommend to replace IABP counterpulsation by the more powerful percutaneous LVAD for the treatment of cardiogenic shock patients who do not respond sufficiently to pharmacologic therapy.

Chapter 19 describes the order of IABP insertion and primary PCI and its association with infarct size and mortality in patients with cardiogenic shock from STEMI. Early IABP insertion before primary PCI might be associated with higher peak creatine kinase levels, indicating a larger infarct size. A possible explanation may be the increased reperfusion delay. The order of IABP insertion and primary PCI was not associated with 30-day and

late mortality. This study suggests that early reperfusion could have priority over routine early IABP insertion in STEMI patients with cardiogenic shock. Randomized studies are needed to determine the optimal timing of IABP insertion relative to primary PCI.

Chapter 20 describes the 30-day and long-term mortality of patients with acute myocardial infarction complicated by cardiogenic shock who were treated with IABP. The 30-day mortality decreased over the years from 52% in 1990-1994 to 36% in 2000-2004. Overall 10-year survival rate was 36%. Cardiogenic shock patients who survived the first 30-days have an encouraging long-term outcome.

Chapter 21 reviews clinical trials on the use of IABP. Most of the performed trials could not demonstrate a clinical benefit of IABP use. However, almost all of the trials were underpowered for the mortality endpoint. Although the recent IABP-SHOCK II trial showed that routine IABP implantation beyond common inotropic support in cardiogenic shock probably has no benefit on reducing mortality, the problem is that there is no proven alternative. We are still awaiting the results of an adequately powered randomized trial of percutaneous left ventricular assist device showing reduction in mortality compared to IABP. In the meantime, we propose a restricted use of IABP in carefully selected patients undergoing high-risk PCI or high-risk patients with persistent, but reversible, circulatory failure despite pharmacological support.

Conclusions and future perspectives

In this thesis, IVUS-VH and NIRS imaging of coronary atherosclerosis appeared to be promising tools for risk prediction in patients with established coronary artery disease. Our study was the first to demonstrate that the presence of thin-cap fibroatheroma lesions on in-vivo IVUS-VH imaging is associated with the occurrence of acute cardiac events. Furthermore, we were the first to demonstrate that the lipid core burden on NIRS is associated with cardiovascular outcome. Before IVUS-VH and NIRS may be used in routine clinical practice, their incremental prognostic value over established cardiovascular risk factors should be evaluated in larger studies with extended follow-up. Meanwhile, IVUS-VH and NIRS measurements may be used as surrogate endpoints in drug development studies.

Blood biomarkers of myocardial necrosis, inflammation, lipid profile and blood coagulation also appeared to be promising tools for risk prediction in patients with established coronary artery disease. Although most of the investigated biomarkers were already known to be related to coronary artery disease, less was known on their association with coronary plaque characteristics. We have demonstrated that most of the biomarkers had an association with at least one of the high-risk coronary plaque characteristics on IVUS-VH or NIRS. Although the investigated blood biomarkers seemed

to have less strong associations with MACE compared to intracoronary imaging, the major advantage of biomarkers is that they can be measured non-invasively and repeatedly over time. Studies on the value of repeated biomarker measurements are warranted. Furthermore, a multiple-biomarker panel may have a higher prognostic value than any single biomarker alone. Moreover, combining clinical covariates, intracoronary imaging and biomarker measurements in a prognostic model may even further improve risk prediction.

In the future, incorporation of intracoronary imaging and/or blood biomarkers as prognostic tools may facilitate clinical decision making and policy applications. It can be hypothesized that high-risk patients may have more benefit from an aggressive treatment strategy with more potent drugs. It is likely that ongoing research will yield such novel, more potent drugs that can slow, or even reverse, the effects of coronary atherosclerosis, for example more potent statins and PCSK9 inhibitors.

Patients with cardiogenic shock from acute myocardial infarction have a high 30-day mortality rate. We found that the 30-day mortality decreased from 52% in 1990 to 38% in 2000, which was most likely related to the implementation of primary PCI as routine treatment of acute myocardial infarction. However, 30-day mortality did not further decrease between 2000 and 2012. Additionally, we found that cardiogenic shock patients who survived the acute phase have a favorable long-term outcome. Therefore, it is important to search for treatment strategies that can lower the mortality rate in the acute phase.

Disappointingly, recent studies have failed to demonstrate a reduction of 30-day mortality with the use of mechanical circulatory assist devices. Several registries and a randomized clinical trial showed that the routine use of IABP did not reduce 30-day mortality. Furthermore, our meta-analysis showed that the use of percutaneous LVAD systems did not reduce 30-day mortality despite their superior hemodynamic support compared to IABP. Nevertheless, we still believe that selected patients with reversible cardiogenic shock and reversible organ damage may have benefit from mechanical circulatory assistance. Larger, adequately powered, randomized controlled trials using IABP, percutaneous LVAD or extracorporeal membrane oxygenation (ECMO) systems are required to assess whether such selected patients may have benefit from mechanical assist devices on 30-day survival.

Meanwhile, we propose a restricted use of IABP and percutaneous LVAD systems in carefully selected patients. Although primary PCI should have priority, mechanical circulatory assistance may be most effective when it is initiated in an early phase when cardiogenic shock is still reversible. Our risk chart that has been developed to assess 30-day mortality risk at time of hospital admission may aid clinical decision making in the acute phase.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 beschrijft de motivering en de opzet van de "European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound" (ATHEROREMO-IVUS) studie. Deze grote, prospectieve, observationele cohort studie heeft het doel om de relatie tussen nieuwe biomarkers en coronaire plaque karakteristieken bepaald met "intravascular ultrasound virtual histology" (IVUS-VH) en "near-infrared spectroscopy" (NIRS) beeldvorming te bestuderen. Verder heeft deze studie het doel om de prognostische waarde van nieuwe biomarkers en van IVUS en NIRS te bestuderen.

Hoofdstuk 2 beschrijft de prognostische waarde van het detecteren van hoog risico coronaire plaques met IVUS-VH in patiënten met coronairlijden. De aanwezigheid van "thin-cap fibroatheroma" laesies (dat geacht wordt het meest voorkomende substraat te zijn van plaque ruptuur leidend tot acuut coronair syndroom) op IVUS-VH is sterk voorspellend voor mortaliteit en het optreden van acuut coronair syndroom. "Thin-cap fibroatheroma" laesies met een grote "plaque burden" hebben een hoger risico dan kleinere laesies, vooral op de korte termijn.

Hoofdstuk 3 beschrijft de prognostische waarde van intracoronaire beeldvorming met NIRS. De "lipid core burden index" (LCBI) dat is bepaald met NIRS in een "non-culprit" bloedvat is geassocieerd met het optreden van cardiale eindpunten binnen 1 jaar in patiënten met coronairlijden die waren verwezen voor coronaire angiografie. Patiënten met een LCBI waarde boven de mediaan van 43 hadden een 4 keer verhoogd risico. Deze bevinding behoeft validatie door grotere studies met een langere follow-up periode.

Hoofdstuk 4 beschrijft de associatie tussen hoog sensitieve troponine-T metingen en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met stabiel coronairlijden. Het volume van de coronaire plaque en de aanwezigheid van "thin-cap fibroatheroma" laesies zijn geassocieerd met een hogere troponine-T waarde in het bloed. De hypothese is dat subklinische plaque rupturen en plaque erosies met distale embolisatie een pathofysiologische mechanisme zou kunnen zijn van de verhoogde troponine waarden, en kan tevens de relatie tussen troponine waarde en cardiale uitkomst verklaren.

Hoofdstuk 5 beschrijft de associatie tussen hoog sensitieve "C-reactive protein" (CRP) metingen en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. CRP is een marker voor coronaire "plaque burden", maar is niet gerelateerd aan de aanwezigheid van "thin-cap

fibroatheroma" laesies en stenoses. CRP waarden >3mg/L zijn voorspellend voor een ongunstige cardiale uitkomst binnen 1 jaar.

Hoofdstuk 6 beschrijft de associatie tussen cytokine waarden in het bloed en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. In patiënten met stabiel coronairlijden is een hogere "tumor necrosis factor-α (TNF-α) waarde geassocieerd met hogere "plaque burden" en de aanwezigheid van "thin-cap fibroatheroma" laesies. Een lagere interleukine-10 waarde is geassocieerd met hogere "plaque burden" en grote "thin-cap fibroatheroma" laesies. Deze bevindingen suggereren dat cytokines een rol spelen in de omvang en vulnerabiliteit van atherosclerose.

Hoofdstuk 7 beschrijft de associatie tussen acute fase eiwitten in het bloed en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. Een hogere ferritine waarde is geassocieerd met een grotere coronaire plaque volume. Een hogere PAI-1 waarde is geassocieerd met mortaliteit het optreden van acuut coronair syndroom. Geen van de onderzochte acute fase eiwitten hadden een consistente associatie met de samenstelling van atherosclerose of vulnerabiliteit van de plaque.

Hoofdstuk 8 beschrijft de associatie tussen chemokine waarden in het bloed en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. "Regulation upon activation normal T-cell expressed and secreted" (RANTES) is een veelbelovende biomarker dat een inverse associatie heeft met coronaire "plaque burden" en plaque vulnerabiliteit, alsook met mortaliteit en het optreden van acuut coronair syndroom.

Hoofdstuk 9 beschrijft de associatie tussen antigenen tegen periodontale pathogenen en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. Plasma IgG en IgA tegen belangrijke periodontale pathogenen waren niet geassocieerd met de omvang van coronaire atherosclerose (met uitzondering van een trend in diabetici) en plaque vulnerabiliteit. IgA tegen periodontale pathogenen had een inverse associatie met coronaire "remodeling". Al met al voegen deze resultaten geen bewijs toe dat een systemische blootstelling aan periodontale pathogenen een substantiële rol speelt in coronairlijden.

Hoofdstuk 10 beschrijft de associatie tussen "proprotein convertase substilisin/kexin type 9" (PCSK9) en coronaire plaque inflammatie gemeten met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. Serum PCSK9 waarden

zijn lineair geassocieerd met het percentage en de absolute hoeveelheid van "necrotic core" weefsel in coronaire atherosclerose en met de klinische uitkomst binnen 1 jaar na coronaire angiografie. Deze associatie was onafhankelijk van serum "low-density lipoprotein" LDL waarde en statine gebruik. PCSK9 zou een interessante therapeutische "target" kunnen zijn in de behandeling van atherosclerose bovenop de LDL regulatie.

Hoofdstuk 11 beschrijft de associatie van 8 eerder geïdentificeerde hoog risico cholesteryl ester, ceramide en lactosylceramide lipiden en 3 ceramide ratio's met coronaire plaque karakteristieken bepaald met IVUS-VH en NIRS, en met klinische uitkomst binnen 1 jaar in een groot cohort van patiënten met coronairlijden. De belangrijkste bevinding is dat hogere concentraties van enkele van de onderzochte lipiden geassocieerd zijn met meer vulnerabele plaque morfologie, uitend in een hogere percentage van de coronaire plaque bestaande "necrotic core" weefsel bij IVUS-VH beeldvorming en een hogere LCBI bij NIRS beeldvorming. Bovendien zijn Cer(d18:1/16:0) en de ceramide ratio's voorspellend voor cardiovasculaire uitkomst binnen 1 jaar.

Hoofdstuk 12 beschrijft de associatie tussen von Willebrand factor (VWF) en coronaire plaque karakteristieken bepaald met IVUS-VH in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. In patiënten met stabiel coronairlijden was een hogere coronaire "plaque burden" geassocieerd met een hogere VWF waarde in het bloed. Hogere VWF waarden waren voorspellend voor een ongunstige cardiovasculaire uitkomst en mortaliteit binnen 1 jaar.

Hoofdstuk 13 beschrijft de prognostische waarde van hoog sensitieve CRP metingen voor cardiovasculaire uitkomst tijdens een follow-up periode van 10 na percutane coronaire interventie (PCI). Hogere CRP waarden gemeten rondom de procedure zijn voorspellend voor mortaliteit en het optreden van myocardinfarcten. Hoewel het toevoegen van CRP in een predictiemodel met traditionele cardiovasculaire risicofactoren de discriminatie niet deed toenemen, was CRP wel is staat om de risico classificatie te verbeteren. Derhalve zou CRP een nuttige biomarker kunnen zijn om de risico inschatting bij patiënten die PCI ondergaan te verbeteren.

Hoofdstuk 14 beschrijft de associatie van 13 plasma biomarkers die eerder zijn geïdentificeerd bij "proteomics" of immunohistochemie experimenten in humane carotide plaques met cardiovasculaire uitkomst in patiënten met coronairlijden die zijn verwezen voor coronaire angiografie. OGN en NGAL/MMP9 zijn veelbelovende biomarkers die tot expressie worden gebracht in vulnerabele atherosclerotische plaques en die voorspellend zijn voor cardiovasculaire eindpunten binnen 1 jaar na coronaire angiografie. Beide

biomarkers verbeterden de risico classificatie in een predictiemodel met traditionele risicofactoren en CRP.

Hoofdstuk 15 beschrijft de associatie tussen de plasma waarden van 42 cytokines, chemokines en groeifactoren gemeten met een "multiplex assay" en cardiovasculaire uitkomst in patiënten met coronairlijden. We vonden dat 3 van de 42 onderzochte biomarkers, namelijk IL18, CXCL8 en CXCL9, mogelijk voorspellers zijn van cardiovasculaire uitkomst in patiënten met coronairlijden.

Hoofdstuk 16 beschrijft de ontwikkeling van een simpele risicotabel voor een vroege inschatting van de 30-daagse mortaliteit in patiënten met cardiogene shock door een ST-elevatie myocardinfarct (STEMI) die zijn opgenomen voor primaire PCI. De 30-daagse mortaliteit was 38.4% en veranderde niet tussen 2000 en 2012. De mortaliteit kan voorspeld worden ten tijde van ziekenhuisopname met behulp van de risicotabel die bestaat uit 3 variabelen, namelijk leeftijd, initiële serum lactaat waarde, en kreatinine waarde. Deze risicotabel is in staat om het mortaliteitsrisico van een patiënt te stratificeren in categorieën van 0-20% tot 80-100%. De risicotabel presteerde beter dan de GRACE risico score.

Hoofdstuk 17 beschrijft de prognostische waarde van sublinguale metingen van de microcirculatie met "sidestream dark field" beeldvorming in patiënten met cardiogene shock door een acuut myocardinfarct. Een verminderde sublinguale microcirculatie op "baseline" en tijdens de behandeling zijn geassocieerd met de ontwikkeling van multiorgaan falen en is een voorspeller voor 30-daagse mortaliteit. Sublinguale meting van de microcirculatie zou overwogen kunnen worden om de ernst van de cardiogene shock te bepalen en om het mortaliteitsrisico van een patiënt in te schatten.

Hoofdstuk 18 is een meta-analyse van gecontroleerde trials die percutane "left ventricular assist devices" (LVADs) met de intra-aortale ballonpomp (IABP) hebben vergeleken in patiënten met cardiogene shock. Het gebruik van een percutane LVAD levert superieure hemodynamische ondersteuning in vergelijking met het gebruik van de IABP. De superieure hemodynamische ondersteuning van de percutane LVADs vertalen zich echter niet in een lagere 30-daagse mortaliteit. Bovendien zijn de percutane LVADs invasiever dan de IABP en leiden tot meer complicaties. Tot op heden kunnen wij nog niet aanbevelen om de krachtigere percutane LVADs te gebruiken in plaats van de IABP ter behandeling van patiënten met cardiogene shock die onvoldoende baat hebben van alleen farmacologische behandeling.

Hoofdstuk 19 beschrijft de associatie van de volgorde van het inbrengen van de IABP en primaire PCI met de infarctgrootte en mortaliteit in patiënten met cardiogene shock door een STEMI. Het initiëren van ondersteuning met IABP vóór de primaire PCI zou geassocieerd kunnen zijn met een hogere piek creatinine kinase waarde dat duidt op een groter infarct. Een mogelijk verklaring is de langere tijd totdat reperfusie optreedt. De volgorde van het inbrengen van de IABP en primaire PCI was niet geassocieerd met de 30-daagse mortaliteit. Deze studie suggereert dat een snelle reperfusie prioriteit zou kunnen hebben boven het initiëren van circulatoire ondersteuning met de IABP. Een gerandomiseerde studie zou de optimale volgorde van het inbrengen van de IABP en primaire PCI moeten uitwijzen.

Hoofdstuk 20 beschrijft de 30-daagse mortaliteit en de mortaliteit op de lange termijn van patiënten met cardiogene shock door een acuut myocardinfarct die zijn behandeld met een IABP. De 30-daagse mortaliteit is afgenomen van 52% in 1990-1994 tot 36% in 2000-2004. De 10-jaars overleving is 36%. Cardiogene shock patiënten die de eerste 30 dagen hebben overleefd hebben een goede uitkomst op de lange termijn.

Hoofdstuk 21 beschouwt de klinische trials die de IABP hebben bestudeerd. De meeste trials hebben geen verlaging in mortaliteit kunnen aantonen. Echter waren bijna alle trials “underpowered” voor mortaliteit als eindpunt. De IABP-SHOCK II trial toonde dat het routinematig gebruiken van een IABP bij cardiogene shock patiënten de mortaliteit niet verlaagd. Het is probleem is echter dat er geen bewezen alternatieven zijn. We wachten nog steeds op de resultaten van een gerandomiseerde trial met voldoende power die aan kan tonen dat het gebruik van een percutane LVAD de mortaliteit verlaagt ten opzichte van de IABP. Intussen stellen wij voor om de IABP beperkt te gebruiken in geselecteerde patiënten die een hoog risico PCI ondergaan en hoog risico patiënten met persisterende, maar nog reversibele, cardiogene shock.

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CURRICULUM VITAE

Jin Ming Cheng was born on July 27th, 1987 in Leidschendam, the Netherlands. After finishing his secondary school at Alfrink College, Zoetermeer, he studied Medicine at Erasmus University Rotterdam. During his study, he started a research project on the management and outcome of cardiogenic shock. He received several awards for his work, including the prestigious Gerrit Jan Mulder prize in 2009 and the Korteweg-Overwater prize in 2010 for the best scientific research performed during medicine study. After obtaining his medical degree (cum laude) in 2011, he started working as a research fellow to Prof. Dr. H. Boersma at Erasmus MC, Rotterdam. He worked on several clinical studies on biomarkers and imaging of coronary atherosclerosis. In parallel to this work, he attended a Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES), where he graduated (cum laude) in 2013. Subsequently, he worked on a collaborative research project on cardiovascular biomarkers at Peking University Clinical Research Institute, Beijing, China, for a period of six months. In October 2014, he became a clinical resident in Cardiology at Erasmus MC, Rotterdam.

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* These authors contributed equally.

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Oral presentations:

Cheng JM, Valk SDA, Den Uil CA, van der Ent M, Lagrand WK, van de Sande M, van Domburg RT, Simoons ML. Usefulness of intra-aortic balloon pump counterpulsation in patients with cardiogenic shock from acute myocardial infarction. Nederlandse Vereniging voor Cardiologie Jubileum Voorjaarscongres 2009, Amsterdam, the Netherlands.

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Cheng JM, Valk SDA, Den Uil CA, van der Ent M, Lagrand WK, van de Sande M, van Domburg RT, Simoons ML. Usefulness of intra-aortic balloon pump counterpulsation in patients with cardiogenic shock from acute myocardial infarction. European Society of Cardiology Congress 2009, Barcelona, Spain.

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Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, Kardys I, Akkerhuis KM, de Boer SPM, Regar E, van Geuns RJ, Serruys PW, Boersma E. High sensitivity C-reactive protein is associated with coronary plaque burden as measured by intravascular ultrasound and adverse cardiovascular outcome. American Heart Association Scientific Sessions 2013, Dallas, Texas, USA.

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Cheng JM, Oemrawsingh RM, Akkerhuis KM, Garcia-Garcia HM, de Boer SPM, van Geuns RJ, Serruys PW, Kardys I, Boersma E. Associations of inflammatory biomarkers with intravascular ultrasound-derived measures of coronary plaque burden and clinical outcome – results of the ATHEROREMO-IVUS study. American College of Cardiology Scientific Sessions 2013, San Francisco, California, USA.

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Cheng JM, Oemrawsingh RM, Akkerhuis KM, Kardys I, Van Geuns RJ, Boersma E, Serruys PW, Van Domburg RT. High-sensitivity C-reactive protein predicts 10-year cardiovascular outcome after percutaneous coronary intervention with drug-eluting stent implantation. American College of Cardiology Scientific Sessions 2014, Washington, DC, USA.

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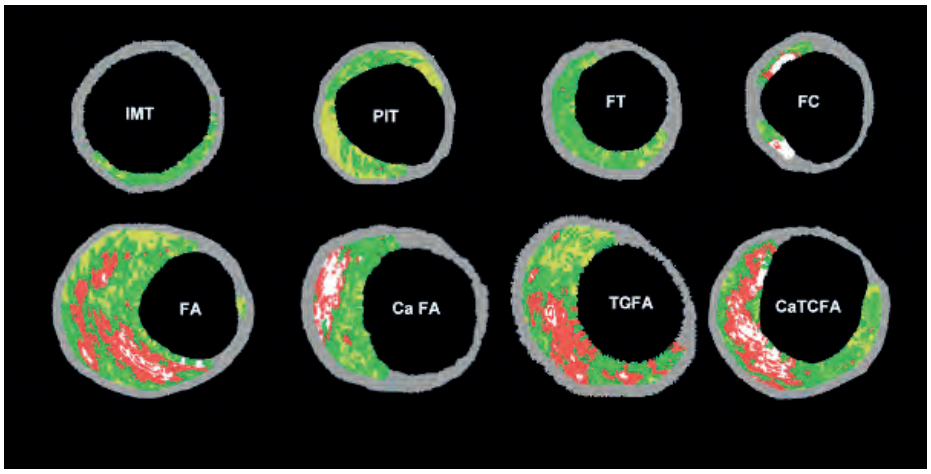
and cardiovascular outcome. American College of Cardiology Scientific Sessions 2014, Washington, DC, USA.

Cheng JM, De Boer SPM, Range H, Garcia-Garcia HM, Akkerhuis KM, Meilhac O, Van Geuns RJ, Serruys PW, Boersma E, Kardys I. Antibodies to periodontal pathogens in relation to coronary plaque characteristics on intravascular ultrasound and to cardiovascular outcome. European Society of Cardiology Congress 2014, Barcelona, Spain.

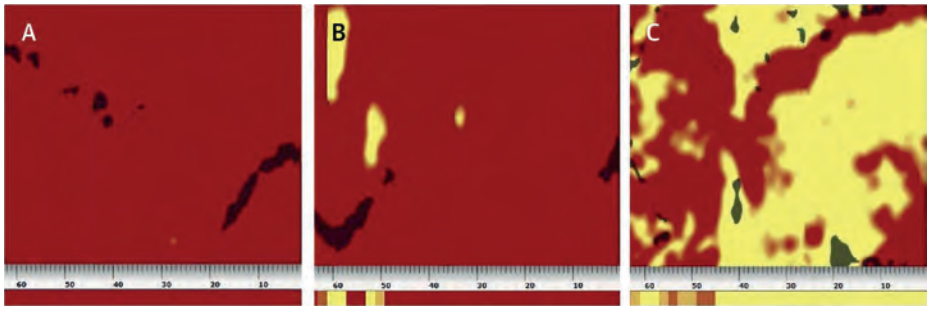
PhD PORTFOLIO

	Year	Workload (ECTS)
1. PhD training		
Research skills		
- BROK course	2012	1.5
- NIHES Master of Science in Clinical Epidemiology	2013	70.0
In-depth courses		
- COEUR Pathophysiology of ischemic heart disease	2012	1.5
- COEUR Cardiovscular imaging and diagnostics	2012	1.5
- COEUR Clinical cardiovascular epidemiology	2012	1.5
- COEUR Atherosclerotic and aneurysmal disease	2012	1.5
- COEUR Arrhythmia research methodology	2012	1.5
- COEUR Intensive care research	2012	1.5
International conferences		
- ESC congress 2009, Barcelona, Spain	2009	1.5
- ACC scientific sessions 2013, San Francisco, CA, USA	2013	1.5
- ESC congress 2013, Amsterdam, the Netherlands	2013	1.5
- AHA scientific sessions 2013, Dallas, TX, USA	2013	1.5
- ACC scientific sessions 2014, Washington, DC, USA	2014	1.5
- ESC congress 2014, Barcelona, Spain	2014	1.5
- AHA scientific sessions 2014, Chicago, IL, USA	2014	1.5
2. Teaching activities		
Lecturing		
- KLEP: Weighted composite endpoints	2012	0.3
- COEUR seminar: Emerging cardiovascular biomarkers	2012	0.3
- Staflunch: Mechanical circulatory support in cardiogenic shock	2012	0.3
- KLEP: Impact of lost to follow-up	2013	0.3
- KLEP: Net reclassification index	2013	0.3
- Staflunch: The vulnerable plaque and beyond	2013	0.3
- Staflunch: Early risk assessment in cardiogenic shock	2014	0.3
Supervising practicals		
- 2nd year medical students: performing a systematic review	2012	0.3
- 2nd year medical students: performing a systematic review	2013	0.3
Presentations		
- ESC congress 2009, Barcelona, Spain	2009	0.3
- ACC scientific sessions 2013, San Francisco, CA, USA	2013	0.3
- ESC congress 2013, Amsterdam, the Netherlands	2013	0.3
- AHA scientific sessions 2013, Dallas, TX, USA	2013	0.3
- ACC scientific sessions 2014, Washington, DC, USA	2014	0.3
- ESC congress 2014, Barcelona, Spain	2014	0.3
- AHA scientific sessions 2014, Chicago, IL, USA	2014	0.3

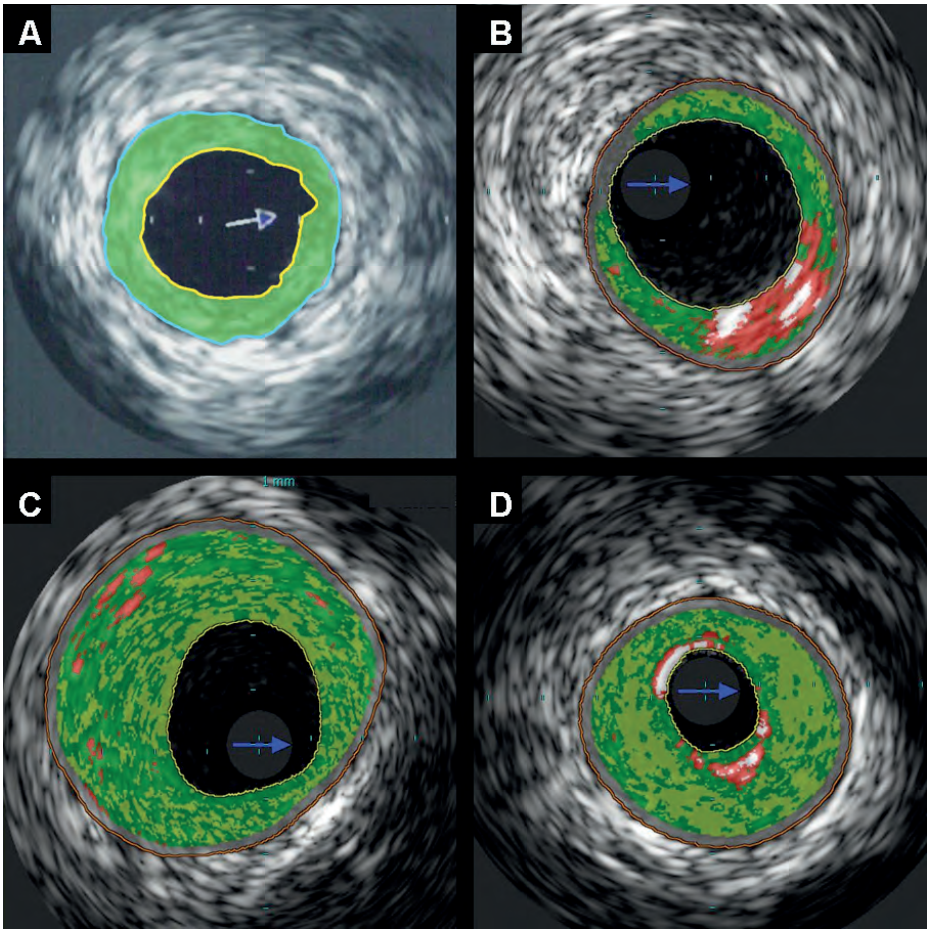
APPENDIX: COLOR FIGURES



Color 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.

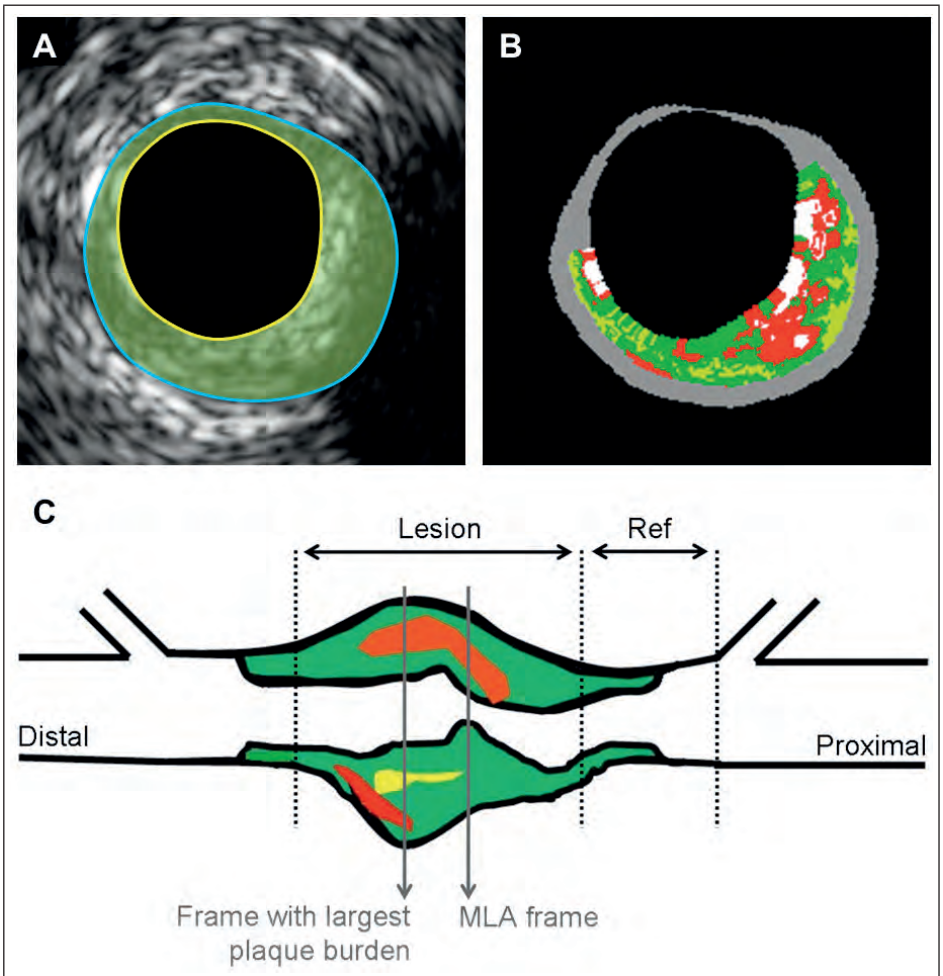


Color figure 2. Intracoronary near-infrared spectroscopy displayed as a chemogram
The figures display the graphical result of coronary wall evaluation with near-infrared spectroscopy in 3 different patients. Spectral characteristics of lipid core– containing coronary plaques (LCP) are displayed along 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).



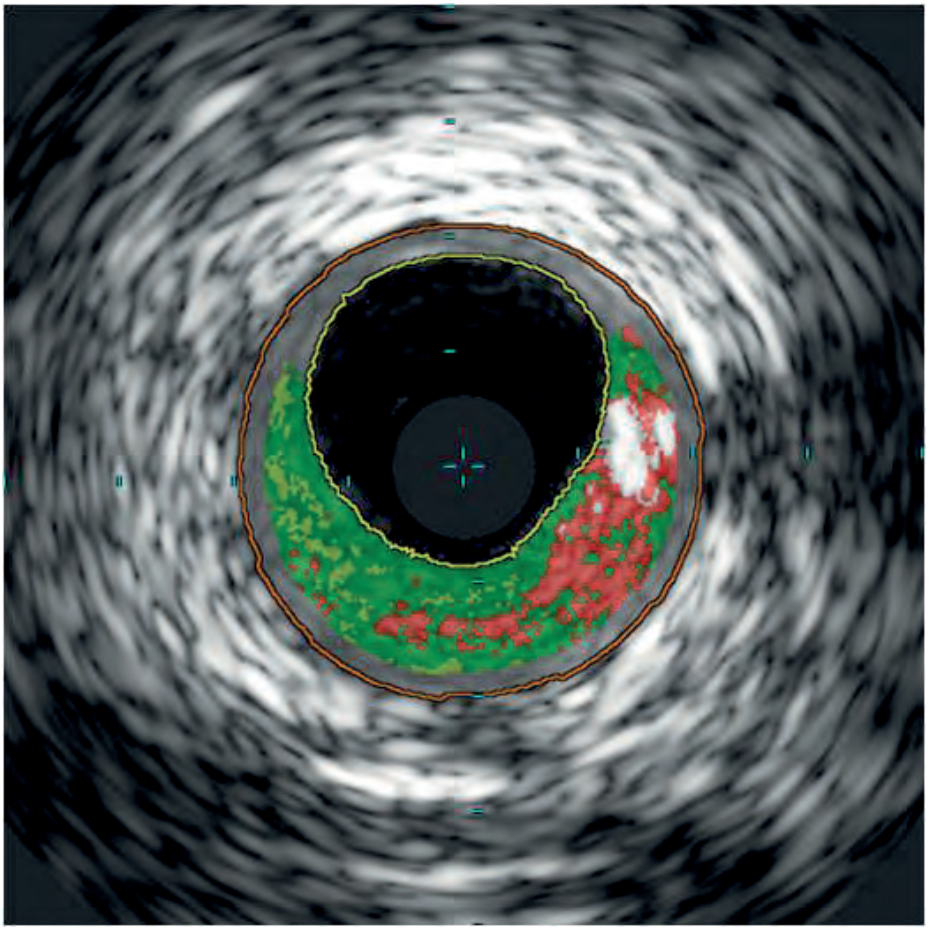
Color figure 3. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound virtual histology

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. C: Lesion with plaque burden of ≥70%. D: Lesion with a minimal luminal area of ≤4.0 mm^2.



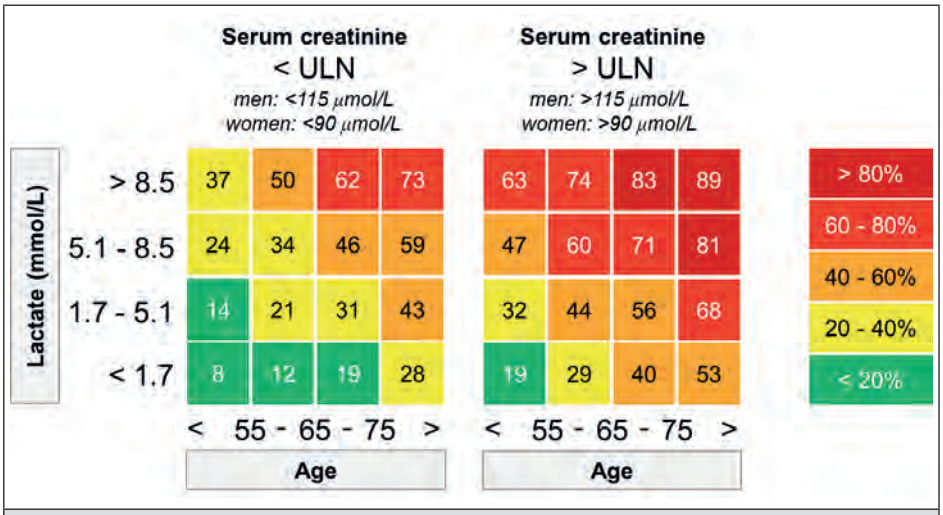
Color figure 4. Measurement of plaque burden, thin-cap fibroatheroma lesions and remodeling index with intravascular ultrasound virtual histology

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. C: A lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Remodeling index was calculated by dividing the external elastic membrane cross-sectional area at the site of minimal luminal area (MLA) by the reference external elastic membrane cross-sectional area. Positive remodeling was defined as a remodeling index of >1.05.



Color figure 5. Characterization of tissue types with 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).



Color figure 6. Risk chart for initial risk assessment of 30-day mortality in cardiogenic shock from ST-elevation myocardial infarction
ULN, upper limit of normal.

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