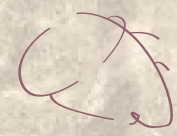
Maxime M. Vroegindewey

THE PROGNOSTIC VALUE OF CORONARY IMAGING AND BIOMARKERS IN ISCHEMIC HEART DISEASE



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The Prognostic Value of Coronary Imaging and Biomarkers in Ischemic Heart Disease

De prognostische waarde van coronaire beeldvorming en biomarkers in ischemische hartziekte

Proefschrift

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Chapter 1

Introduction

Although major advancements have been achieved in prognostication and treatment of patients with atherosclerosis, cardiovascular (CV) disease (CVD) remains the number one cause of death globally. In Europe alone, every year 3.9 million people die from the effects of atherosclerosis (Figure 1).[1]

Atherosclerosis is an acquired chronic condition, which may cause ischemic heart disease (IHD) including stable angina pectoris (SAP) or acute coronary syndrome (ACS), the collective term for unstable angina pectoris, non ST-elevation myocardial infarction and ST-elevation myocardial infarction. Patients who are diagnosed with IHD are at high risk of developing (recurrent) CV events. Moreover, patients who experience a recurrent CV event are 2.5 times more likely to die of that event, than first-time experiencers.[2] Within the first year after hospital discharge, the death rate in IHD patients is as high as 9%.[3] Without proper treatment, the annual death rate remains on average 5% hereafter, resulting in a cumulative death rate of almost 80 percent 15 years post hospital discharge in IHD patients (Figure 2).[3]

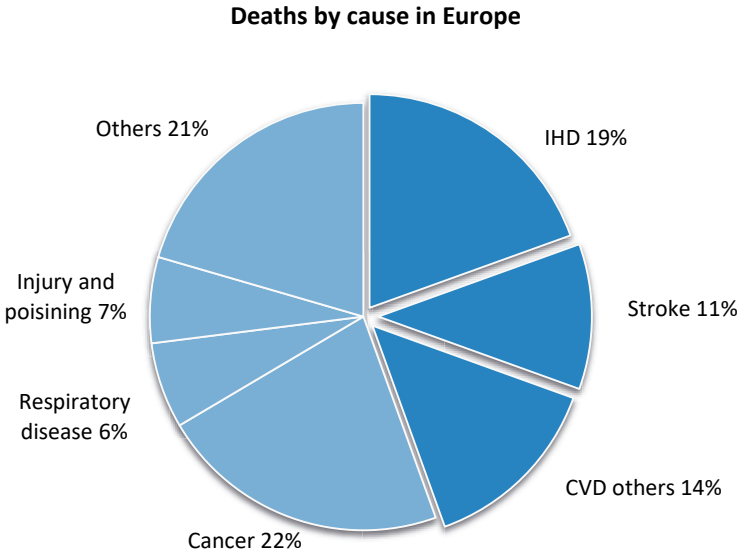

Figure 1. Causes of deaths in Europe by the European Cardiovascular Disease Statistics 2017

Currently, over 30 million people in Europe have established IHD, and, thus, are prone to recurrent CV events and cardiac death.[1] In recent decades, several clinical risk factors have been identified that advance pathological progression of atherosclerosis, such as diabetes mellitus, hypertension, hyperlipidaemia and smoking.[4] These factors may be used to foster tailored treatment and monitoring during clinical follow-up. However, clinical risk factors do not reflect the actual coronary atherosclerotic burden of a patient, nor are they a proxy for dynamic atherosclerotic changes. To further improve

risk stratification of IHD patients, one should aim to effectuate a more precision-based approach to prognostication, and, eventually, to secondary preventive care in IHD patients. In particular, imaging modalities and circulation biomarkers may be of interest in this context.

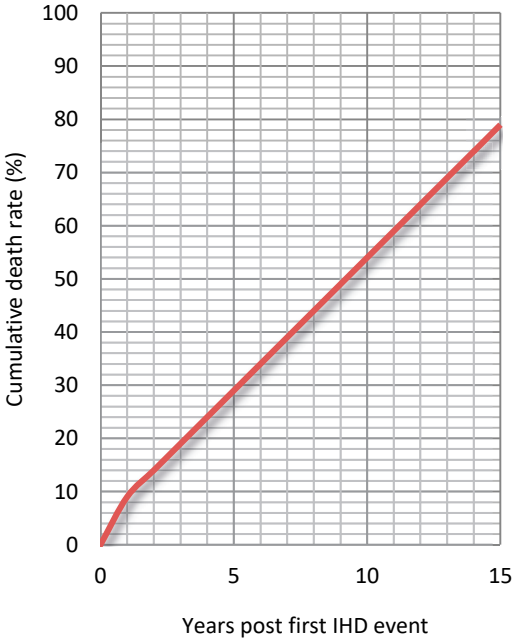

Figure 2. Cumulative death rate after hospital discharge in patients with IHD*
*As described in *Law et al*.(3)

Atherosclerosis is a lipid driven inflammatory disease. The pathophysiology of the onset and progression of atherosclerosis is complex. Although atherosclerosis manifests itself in various forms, some key inflammatory processes have been established in the development and progression of atherosclerotic plaques in IHD.

Atherosclerotic plaque formation

In general, atherosclerotic plaques tend to form at coronary artery sites with disrupted blood flow, such as in curvatures and bifurcations of branches, where the vascular wall shear-stress is distributed irregularly. Constant wall shear-stress is a requisite for well-functioning stable endothelial cells and stimulates atheroprotective pathways.[5] In case of disturbed wall shear-stress, flow-initiated inflammatory factors are activated in the endothelium and may change endothelial cells in dysfunctional cells. These cells may induce multiple processes associated with the onset of atherosclerotic plaque.

First, one of these early processes associated with plaque formation is the reduction in nitric oxide production by the dysfunctional endothelium.[6] Nitric oxide is involved in various atheroprotective processes, and one of its major functions is endothelium-dependent vasodilatation. Dysregulation of the vascular tone may induce vasospasms and early ischemia.

Secondly, it has been established that the permeability of the endothelial wall changes during the onset of plaque formation.[7, 8] The increasing permeability of the endothelial wall may induce influx of extracellular macromolecules. In the presence of high blood concentrations of lipoproteins, this may result in the migration and accumulation of lipoproteins in the endothelial wall. Sub-endothelial cell accumulation may be accompanied by the recruitment of immune cells such as monocytes and T-lymphocytes.[8] Under normal conditions, monocytes migrate into the endothelial wall to decrease further entry of inflammatory cells and to differentiate in macrophages to absorb lipoproteins and efferocytose apoptotic cells.[9] However, under disturbed conditions, monocytes will accumulate increasingly, and fail to both reduce entry of other cells and remove abundant cells. Moreover, T-lymphocyte recruitment continues, promoting further inflammation.

Lastly, under these disturbed conditions, the dysfunctional endothelium may upregulate the expression of adhesion molecules, facilitating the increasing sub-endothelial accumulation of lipoproteins and inflammatory cells.[8] Together, these processes precipitate the formation of an atherosclerotic plaque (Figure 3).

Atherosclerotic plaque progression

The progression of a formed plaque is associated with sub-endothelial foam cell formation. Foam cell formation is initiated by oxidants produced by the dysfunctional endothelium. These oxidants modify sub-endothelial low-density lipoproteins (LDL).[10, 11] Subsequently, accumulated macrophages will absorb these modified LDL molecules and turn into fat-laden macrophages called foam cells. Foam cells are prone to apoptosis and necrosis. Moreover, the foam cell production process is associated with increased dysregulation of the sub-endothelial lipid metabolism and impairs the removal of cholesterol molecules by macrophages from the endothelial wall. [10, 11] Altogether, this process is responsible for increasing sub-endothelial lipoprotein accumulation and the formation of a necrotic core. At the same time, the chronic inflamed endothelium increases recruitment of monocytes and T-cells, advancing inflammatory exacerbation (Figure 3).[8]

In reaction to these plaque destabilising processes, smooth muscle cells of the media may migrate to the intima of the artery wall.[12] These smooth muscle cells can produce extra cellular matrix proteins which, in turn, stabilise the plaque by forming a fibrous cap around the atherosclerotic plaque cells. In addition, migrated smooth muscle cells may undergo apoptosis and calcify, which adds stability to the plaque.[13] Consequently, an

advanced plaque may be characterised by chronic inflammation, abundance of foam cells, necrotic core, smooth muscle cell infiltration, calcification, and a fibrous cap.

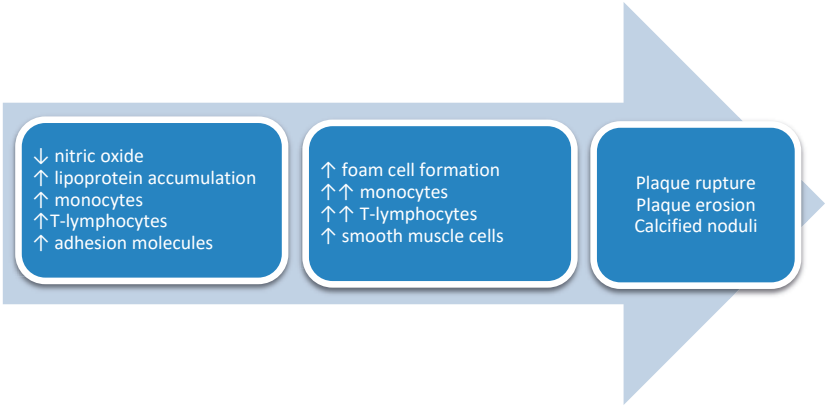

Figure 3. Brief overview of the processes underlying atherosclerotic plaque progression

Atherosclerotic plaque rupture

A stable advanced plaque is characterised by a robust fibrous cap that is able to resist the vascular shear-stress on the endothelial wall, separating the atherosclerotic plaque cells from the blood stream.[14] However, if the integrity of the cap weakens, an advanced plaque may become vulnerable and rupture (Figure 3).[15] In response, atherosclerotic plaque cells will excrete large amounts of tissue factor, stimulating thrombus formation.[16] In addition, fibrinolytic pathways responsible for the stability of a thrombus will be activated.[14] a thrombus can subsequently partly or completely occlude a coronary artery, and cause ischemia and subsequent myocardial infarction.

In addition to the mechanism of plaque rupture described above, plaque erosion or calcified noduli of a stable plaque extending in the lumen of a coronary artery may also result in thrombosis causing ischemia and subsequent ACS.[17]

Coronary imaging and biomarkers in ischemic heart disease

As mentioned previously, to further improve risk stratification in IHD patients, parameters that feature the coronary atherosclerotic burden of these patients, and that reflect the dynamics of plaque progression are warranted. Potentially, these parameters may effectuate a more precision-based approached to prognostication in IHD patients. In this context, invasive imaging modalities such as coronary angiography (CAG) or intracoronary imaging modalities such as infra-red spectroscopy (NIRS) and intra-vascular ultrasound (IVUS) may be of pivotal value. NIRS and IVUS can be used to quantify and qualify coronary atherosclerotic burden. NIRS is applied to measure the intracoronary lipid content of atherosclerosis by creating a chemogram of the coronary wall and expressing the detected amount of lipid tissue in a lipid core burden index (LCBI).[18]

Grayscale IVUS is used to quantify total intracoronary plaque volume and burden and radiofrequency IVUS is used to qualify plaque components as fibrous, fibro-fatty, necrotic core and dense calcium tissue.[19, 20] Information on plaque characteristics might provide insights on the degree of a patient's coronary atherosclerotic disease and the risk for future cardiac events.

In addition, circulation biomarkers are of interest, since they may serve as a proxy for atherosclerotic disease progression. A circulation biomarker is any substance measurable in the blood that influences or reflects changes in disease. Although the key mechanisms of plaque progression might be similar among patients, the actual triggers responsible for an event may differ.[21] Therefore, we need to identify (novel) biomarkers involved in inflammatory pathways that advance plaque progression.

Thesis outline

In this thesis we assess the prognostic value of invasive imaging modalities and circulation biomarkers in patients with established IHD. In the first part, we study plaque extent and characteristics of patients admitted to the hospital with SAP or ACS undergoing CAG or percutaneous coronary intervention (PCI) and their follow-up. First, we assess in these patients at baseline the relationship between the SYNTAX score, an validated anatomical CAG-based prediction tool for long-term mortality, and coronary wall pathology as measured by NIRS and IVUS. Secondly, we assess in these patients the long-term prognostic value of NIRS and IVUS. Lastly, we study the performance of the SYNTAX score II, the scoring tool incorporating both the anatomical-based SYNTAX score as well as clinical characteristics to predict long-term mortality in the patients admitted with one- or two-vessel disease.

In the second part of this thesis, we assess in detail the temporal patterns of various circulation biomarkers in patients admitted to the hospital with ACS, using repeated measurements during one year follow-up. As such, we are able to study the trajectory of these biomarkers after an ACS and prior to the development of recurrent CV events during follow-up. Moreover, we are able to assess the prognostic value of repeatedly measured circulation biomarkers, and may identify novel (inflammatory) modulators of recurrent CV events. In addition, we describe the intra-individual LDL variation during one year, in statin-treated post-ACS patients. Lastly, we establish in a systematic review whether auto-antibodies to oxidized LDL molecules are associated with the degree of coronary artery disease of a patient as quantified by CAG, NIRS or IVUS, and if these auto-antibodies are associated with coronary events in patients with and without prevalent IHD.

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Chapter 2

SYNTAX score in relation to intravascular ultrasound and near-infrared spectroscopy for the assessment of atherosclerotic burden in patients with coronary artery disease

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ABSTRACT

Aims

To examine the relationship between the anatomical SYNTAX score (SXscore), derived from all three coronary arteries, and coronary wall pathology measured by radiofrequency-intravascular ultrasound (RF-IVUS) and near-infrared spectroscopy (NIRS) in a single non-culprit segment.

Methods and results

In patients referred for coronary angiography (N=88) or PCI (N=592) for stable angina or acute coronary syndrome, the SYNTAX score calculator (www.syntaxscore.com) was used to determine SXscore before PCI, if applicable. RF-IVUS and/or NIRS were performed in a non-stenotic 40 mm study segment following the clinically indicated angiography/PCI. After adjustment for multiple confounders, a higher SXscore was associated with higher segmental plaque volume in the study segment (2.21 mm3 per SXscore point, 95%CI 0.92-3.50, p-value 0.001), as well as with higher volume of fibrous (0.93 mm3 per point) and fibro-fatty tissue (0.29 mm3 per point). A higher SXscore was also associated with a higher NIRS-derived lipid core burden index in the full study segment (1.35 units per SXscore point, 95%CI 0.22-2.47, p-value 0.019). Importantly, SXscore correlated with the fatty/fibro-fatty and LCBI signals despite adjusting for plaque burden.

Conclusions

In patients with CAD, higher SXscores are associated with higher atherosclerotic burden as assessed by RF-IVUS and NIRS in a single non-stenotic coronary artery segment.

INTRODUCTION

The SYNTAX score (SXscore) is an angiographic tool that grades the complexity of coronary artery disease (CAD) and is also used for short- and long-term prediction of major adverse cardiac events (MACE) in patients undergoing percutaneous coronary intervention (PCI) and/or coronary artery bypass graft surgery (CABG).[1-2] The severity and composition of coronary atherosclerosis as assessed by (radiofrequency-)intravascular ultrasound (RF-IVUS) and near-infrared spectroscopy (NIRS) in *one* (non-)stenotic coronary artery segment have recently also shown prognostic value for MACE.[3-8] Furthermore, RF-IVUS and NIRS in one (non-)stenotic coronary artery segment have previously been used to evaluate the effects over time of anti-atherosclerotic therapy under the assumption that these assessments are representative of the total coronary atherosclerotic burden.[9] However, it has never been investigated in a large cohort how well the atherosclerotic burden as graded by NIRS and RF-IVUS measured in one (non-) stenotic coronary artery segment correlates with the atherosclerotic burden as assessed by the SXscore which is derived from all three coronary arteries.

It is important to realize that the three methods differ from each other in the assessment and quantification of CAD. The SXscore is an anatomical scoring tool that grades luminal coronary obstruction, directly from the coronary angiography (CAG). Therefore, it lacks detail with respect to coronary artery wall pathology. Conversely, RF-IVUS and NIRS have been shown to provide information on plaque morphology in the imaged coronary segment. However, both of these imaging techniques require additional intracoronary catheters, whereas the SXscore itself does not require instrumentation of the coronary lumen.

The aim of this study is to examine the relationship between the coronary atherosclerotic burden measured as luminal coronary obstruction graded by the SXscore, derived from all three coronary arteries, and the atherosclerotic burden by assessing coronary artery wall pathology measured by RF-IVUS and NIRS in one non-stenotic segment of a single non-culprit coronary artery.

METHODS

Study population

This study constitutes a combined analysis of two cohorts: The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis-IVUS (ATHEROREMO-IVUS) study and the Integrated Biomarker and Imaging Study-3 (IBIS-3). The design of both studies has been described elsewhere.[9-11] In total, 770 patients with an indication for diagnostic CAG and/or PCI due to either stable angina pectoris

(SAP) or an acute coronary syndrome (ACS) were included and had a RF-IVUS and/or NIRS performed in a non-stenotic segment of a non-culprit coronary artery.

Both studies were approved by the Medical Ethics Committee of the Erasmus MC and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all included patients. ATHEROREMO-IVUS is registered in ClinicalTrials.gov, number NCT01789411, and IBIS-3 is registered in The Netherlands trial register, number NTR2872.

Coronary intravascular ultrasound and near-infrared spectroscopy

RF-IVUS and NIRS methods have been described in detail previously.[9-11] For a comprehensive methods section, refer to the *Supplementary Methods*.

SYNTAX Score

The SXscore was calculated (pre-PCI) for every CAG taken at study entry using the SYNTAX Score calculator (www.syntaxscore.com). Details concerning the calculation of the SXscore have been described elsewhere.[1] In brief, the three coronary arteries are divided in 16 segments, each with a corresponding weighting factor. If there is a lesion producing 50% or more luminal obstruction, the weighting factor is added. Moreover, other factors that reflect the severity of the atherosclerotic lesion and the possible difficulty of a percutaneous treatment, for example lesion length and diffuse disease of the vessel, are taken into account. Eventually, all points are summed to obtain the SXscore reflecting the complexity of the CAD of the patient.

As applied in other all-comers and ST-segment elevation myocardial infarction (STEMI) populations, lesions caused by in-stent restenosis were considered as de novo lesions.[12-14] Occlusions in patients presenting with ACS were scored as occlusions of unknown duration, as the analyst was blinded to all other patient information.[15]

In case of a codominant coronary artery circulation, the vessel mainly responsible for the perfusion of the posterior side of the heart was designated as the dominant coronary artery. Last, patients with a pre-existing CABG, whose CAG is unquantifiable using the SXscore, were excluded.

The SXscores were determined by a trained analyst who was blinded with respect to other patient characteristics and clinical outcome.

Statistical analysis

Categorical variables are presented as numbers and percentages. The distribution of the continuous variables, including RF-IVUS and NIRS parameters, was examined for normality with Kolmogorov-Smirnov tests. Normally-distributed continuous variables are presented as mean ± standard deviation(SD). Non-normally distributed continuous variables are presented as median and interquartile range(IQR). SXscores were

categorized into tertiles based on the distribution of the SXscores in the particular group that was being examined. Kruskal-Wallis tests were used for multiple group comparison of continuous variables. Categorical variables were compared using Pearson Chi-square tests or Fisher-Freeman-Halton Exact tests when appropriate.

Linear and logistic regression analyses were applied to evaluate the relation between SXscore (explanatory) and RF-IVUS- and NIRS-derived (dependent) variables. Variables concerning plaque volume were first normalized for the imaged segment length (i.e. normalized plaque volume=plaque volume / imaged segment length*median segment length of study population). In multivariable analyses, age, gender, hypertension, renal impairment, hypercholesterolemia, diabetes mellitus, smoking, indication for CAG, history of PCI, as well as segmental plaque burden were entered as potential confounders/explanatory factors. Thus, the models allow to conclude on the relation between SXscore and the RF-IVUS/NIRS imaging signals, irrespective of the patient's segmental plaque burden. Assumptions underlying linear regression models were evaluated by visual examination of the residuals.

All statistical tests were 2-tailed and p-values <0.05 were considered significant. SPSS, Version 21.0 (IBM Corp., Armonk, NY, USA) was used for all the analyses.

RESULTS

Baseline characteristics

The current study included 680 patients from the combined ATHEROREMO-IVUS and IBIS-3 cohorts (Figure 1). The overall SXscore ranged from 0 to 37.5 with a median of 7 (IQR:3-13) and a mean of 8.6±7.4. Baseline clinical and angiographic variables were stratified according to tertiles reflecting the obtained Sxscores (lowest tertile, ≤4; middle tertile, >4 to ≤10; highest tertile >10, Table 1). The highest tertile comprised more men. As expected, more patients in the higher tertiles exhibited 2-or 3-vessel disease, whereas no significant stenosis or 1-vessel disease was more frequently present in patients with the lowest SXscores. More patients with lower SXscores had previously undergone a PCI.

Coronary intravascular ultrasound in relation to SXscore

After adjustment for multiple confounders/explanatory factors, a higher SXscore was associated with a higher plaque volume in the study segment (2.21 mm^3 per SXscore point, 95%CI 0.92-3.50, p-value 0.001) (Table 2). The relation between SXscore and plaque burden was consistent with this observation, although statistically non-significant (p-value 0.078). A higher SXscore was also associated with a higher volume of fibrous (0.93 mm^3 per SXscore point, 95%CI 0.53-1.33, p-value <0.001) and fibro-fatty tissue

(0.29 mm^3 per SXscore point, 95% CI 0.17-0.42, p-value <0.001) (Tables 2, Figure 2). Importantly, the SXscore correlated with the fatty/fibro-fatty signals despite adjusting for plaque burden. In contrast, we found no association between SXscore and necrotic core volume (p-value 0.16) or the presence of TCFA (p-value 0.46).

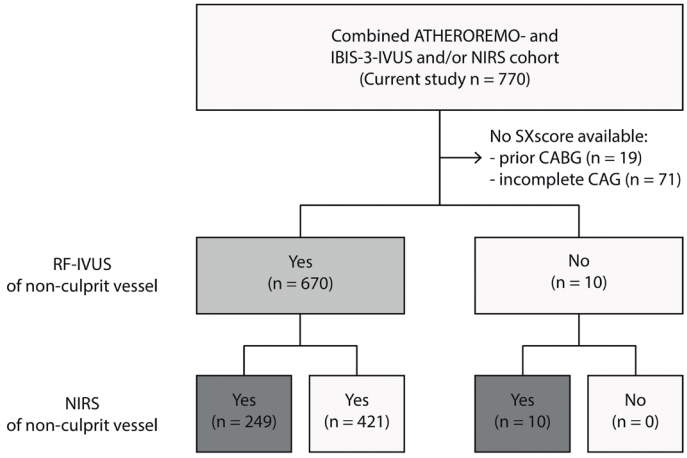

Figure 1. Patient inclusion

RF-IVUS is available in 670 patients(light grey) and NIRS is available in 259 patients(dark grey).
ATHEROREMO-IVUS: The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis-IVUS study, IBIS-3:Integrated Biomarker and Imaging Study-3, NIRS: near-infrared spectroscopy
RF-IVUS: radiofrequency intravascular ultrasound, SXscore: SYNTAX score

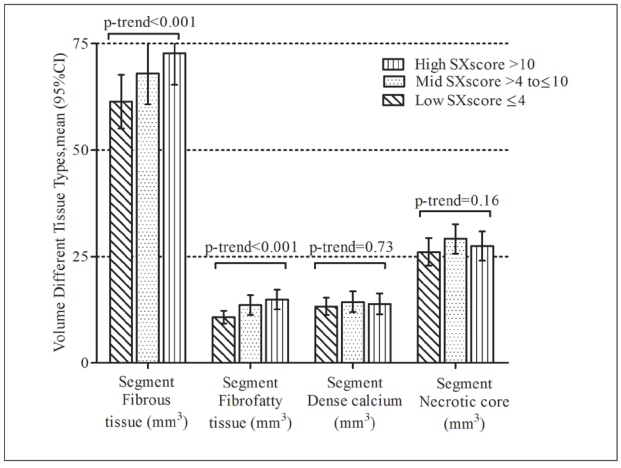

Figure 2. Distribution of RF-IVUS derived plaque components across the SXscore categorized in tertiles

The mean volumes of the different plaque components: fibrous, fibro-fatty, dense calcium and necrotic tissue are divided across the SXscore categorized in tertiles (cut-off points 4 and 10).
RF-IVUS: radiofrequency intravascular ultrasound SXscore: SYNTAX score

Table 1. Baseline characteristics

	Low SXscore ≤4 (n=236)	Mid SXscore >4 to ≤10 (n=221)	High SXscore >10 (n=223)	p-value
Patient characteristics				
Age, years	60.4±10.9	61.3±10.7	61.2±11.0	0.70
Men, n(%)	169(71.6)	169(76.5)	184(82.5)	0.022
Risk factors				
Diabetes Mellitus, n(%)	48(20.3)	42(19.0)	40(17.9)	0.79
Hypertension, n(%)	130(55.1)	130(58.8)	107(48.0)	0.064
Hypercholesterolemia, n(%)	119(50.4)	138(62.4)	118(52.9)	0.028
Smoking, n(%)	63(26.7)	66(29.9)	70(31.4)	0.55
History				
Positive family history, n(%)	138(58.5)	117(52.9)	101(45.3)	0.018
Previous myocardial infarction, n(%)	79(33.5)	59(26.7)	61(27.4)	0.21
Previous PCI, n(%)	94(39.8)	63(28.5)	52(23.3)	<0.001
Previous stroke, n(%)	15(6.4)	12(5.4)	15(6.7)	0.84
History of peripheral artery disease, n(%)	12(5.1)	19(8.6)	14(6.3)	0.31
History of renal insufficiency, n(%)	13(5.5)	10(4.5)	11(4.9)	0.89
History of heart failure, n(%)	8(3.4)	4(1.8)	5(2.2)	0.56
Procedural characteristics				
Indication for coronary angiography				0.001
Stable angina, n(%)	130(55.1)	95(43.0)	88(39.5)	
Acute coronary syndromes, n(%)	106(44.9)	126(57.0)	135(60.5)	
Extent of coronary artery disease				<0.001
No significant stenosis, n(%)	91(38.6)	1(0.5)	0(0.0)	
1-vessel disease, n(%)	134(56.8)	138(62.4)	72(32.3)	
2-vessel disease, n(%)	11(4.7)	69(31.2)	114(51.1)	
3-vessel disease, n(%)	0(0.0)	13(5.9)	37(16.6)	
Imaged coronary artery characteristics				
Imaged segment length, mm	44.7±14.1	42.6±13.1	44.4±14.7	0.17
Imaged coronary artery for RF-IVUS				0.004
Left anterior descending, n(%)	107(46.7)	76(34.5)	64(28.8)	
Left circumflex, n(%)	58(25.1)	78(35.6)	81(36.5)	
Right coronary artery, n(%)	65(28.1)	65(29.9)	76(34.6)	
Imaged coronary artery for NIRS				0.003
Left anterior descending, n(%)	40(44.4)	34(39.1)	20(24.4)	
Left circumflex, n(%)	22(24.4)	36(41.4)	31(37.8)	
Right coronary artery, n(%)	28(31.1)	17(19.5)	31(37.8)	

CABG: coronary artery bypass graft, IQR: interquartile range, LCBI: Lipid Core Burden Index, NIRS: near-infrared spectroscopy, PCI: percutaneous intervention, RF-IVUS:(radiofrequency) intravascular ultrasound, SXscore: SYNTAX score

Table 2. Associations between the SYNTAX score and RF-IVUS and NIRS derived variables in multivariable analyses

	Mean/OR (95%CI)			β/OR† (95% CI)	
RF-IVUS derived variables	SXscore ≤ 4	4 < SXscore ≤ 10	Sxscore > 10		p-value†
No. of patients	230	219	221	670	
Plaque volume, mm^3 ‡	230.1 (215.7-244.4)	246.0 (222.3-269.6)	242.4 (228.8-276.1)	2.21 (0.92-3.50)	0.001
Plaque burden, %‡	38.1 (36.8-39.5)	38.8 (36.7-40.9)	39.0 (37.0-41.1)	0.10 (-0.011-0.22)	0.078
Plaque composition					
Fibrous, mm^3	61.4 (55.1-67.7)	68.0 (60.7-75.4)	72.6 (65.3-80.2)	0.93 (0.53-1.33)	<0.001
Fibro-fatty, mm^3	10.8 (9.3-12.3)	13.6 (11.3-15.9)	14.9 (12.6-17.2)	0.29 (0.17-0.42)	<0.001
Dense calcium, mm^3	13.3 (11.3-15.3)	14.3 (11.9-16.8)	13.9 (11.4-16.3)	0.023 (-0.11-0.16)	0.73
Necrotic core, mm^3	26.1 (22.8-29.3)	29.2 (25.7-32.6)	27.5 (24.0-30.9)	0.14 (-0.52-0.33)	0.16
Lesion morphology					
TCFA	1	0.97 (0.63-1.50)	0.81 (0.53-1.24)	0.99 (0.97-1.01)	0.46
MLA ≤4.0mm^2 ‡	1	0.74 (0.44-1.23)	0.95 (0.59-1.54)	1.00 (0.97-1.02)	0.80
Plaque burden ≥70%‡	1	1.05 (0.58-1.88)	1.47 (0.85-2.53)	1.02 (1.00-1.05)	0.092
NIRS-derived variables	SXscore ≤ 3	3 < SXscore ≤ 8	SXscore > 8		
No. of patients	90	87	82	259	
LCBI region of interest	39.4 (27.9-50.8)	56.0 (36.2-75.9)	62.7 (42.6-82.9)	1.35 (0.22-2.47)	0.019
LCBI worst 10 mm	118.1 (90.9-145.3)	150.3 (106.4-194.3)	176.9 (132.2-221.6)	2.89 (0.39-5.38)	0.024
LCBI worst 4 mm	190.4 (154.6-226.2)	231.3 (175.7-286.5)	266.6 (210.2-323.1)	3.83 (0.69-6.97)	0.017

We present means and odds ratios with 95%CI based on multivariable models with SX score included as categorical (explanatory) variable. In addition, we present β's and odds ratios with SXscore included as continuous (explanatory) variable. Multivariable models are adjusted for age, gender, hypertension, renal impairment, hypercholesterolemia, diabetes mellitus, smoking, indication for CAG, history of PCI and plaque burden.

† Based on multivariable models with SXscore included as continuous (explanatory) variable

‡ Multivariable model without adjustment for plaque burden

CI: confidence interval, LCBI: lipid core burden index, MLA: minimum luminal area, NIRS: near-infrared spectroscopy, No: number, OR: odds ratio, RF-IVUS: radiofrequency intravascular ultrasound, SXscore: SYNTAX score, TCFA: thin-cap fibroatheromas

Near-infrared spectroscopy in relation to SXscore

A higher SXscore was associated with a higher NIRS-derived lipid core burden index(LCBI) in the full study segment (1.35 units per SXscore point, 95%CI 0.22-2.47, p-value 0.019) (Tables 2, Figure 3). Consistent results were observed for the 10 and 4 mm segments with highest LCBI values. Again, it is relevant to note that the observed correlation between SXscore and LCBI signals was independent of segment plaque burden.

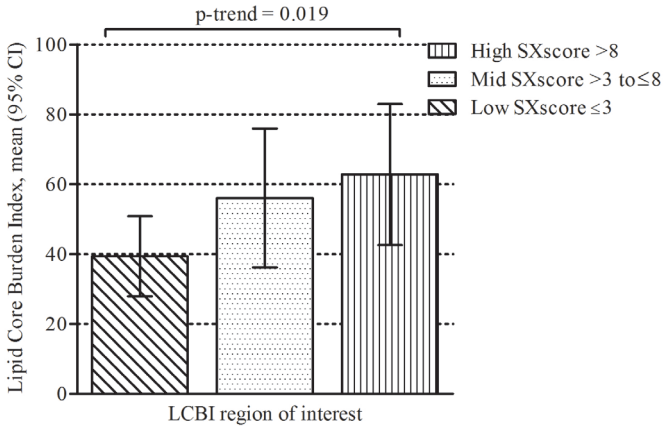

Figure 3. Distribution of NIRS-derived LCBI across the SXscore categorized in tertiles
The mean LCBI of the region of interest is divided across the SXscore categorized in tertiles (cut-off points 3 and 8). LCBI: lipid core burden index, SXscore: SYNTAX score

DISCUSSION

This is the first study, to our knowledge, that systematically examined a large patient population for the correlation of coronary atherosclerotic burden as determined by the SXscore and the extent and characteristics of coronary atherosclerosis as assessed by RF-IVUS and NIRS in one non-stenotic segment of a single non-culprit coronary artery. This study shows that there is a significant and independent association between these entities in patients with CAD.

The SXscore is a well-established angiographic tool for the assessment of the severity and complexity of CAD.[2] It not only evaluates the number of significant stenoses but also lesion length and amount of calcification, amongst others. Still, as the SXscore is based on coronary luminography, it is limited in the assessment of the extent of (non-stenotic) plaque burden and plaque morphology, including the identification of high-risk plaque characteristics and vulnerable plaques. We demonstrated that the SXscore is associated with RF-IVUS and NIRS derived information on the extent and composition of coronary atherosclerosis in patients with CAD. The correlation between SXscore and the amount of fatty/fibro-fatty tissue as well as LCBI were most striking. In this respect it is relevant to note the absence of relations between SXscore and plaque phenotype (necrotic core volume) and lesion morphology (TCFA).

Previously, a significant relation between atherosclerotic burden in one non-culprit coronary segment as assessed by RF-IVUS or NIRS and cardiovascular outcome was demonstrated which persisted after exclusion of culprit-related and imaged segment-related cardiac events.[5,7] This indirectly supported the assumption that the atherosclerotic

burden in one non-culprit coronary segment may be representative for the atherosclerotic disease of the entire coronary tree. The current study shows a direct association between angiographic atheroma burden of all three vessels and intravascular coronary wall evaluation of a non-culprit segment.

Although pre-specified high-risk plaque phenotypes (TCFA, MLA≤4.0mm^2 and lesions with a plaque burden of ≥70%) were not significantly associated with an increase in SXscore, the volume of fibrous and fibro-fatty tissue in plaques was higher in patients with a higher SXscore. Although a previous study has shown that plaque morphology, as measured by three-vessel imaging by optical coherence tomography (OCT) or IVUS, is associated with and may be used for the identification of vulnerable plaques in patients with ACS,[19] it appears from our study that it is the amount of tissue type which is associated with SXscore and not plaque morphology (the layout of the tissue) per se. In light of the relatively overall low angiographic burden of disease in our population, however, it needs to be considered that this finding may not be applicable in a patient population with more advanced CAD. Moreover, necrotic core and dense calcium did not show a significant association with a higher SXscore.

Previously, in one other small cohort, the relationship between NIRS and SXscore was explored but no association was found.[17] The relationship between NIRS and the SXscore has also been studied in a subset of patients from ATHEROREMO-IVUS.[18] The enrichment of the ATHEROREMO-IVUS cohort with the IBIS-3 cohort in the current study substantially increases the sample size and creates more robust data.

In most studies, SXscore is stratified in tertiles or even quartiles reflecting the distribution of the scores found in the respective cohort.[2] The thresholds of the original SYNTAX trial (cut-off points:22 and 33) have been incorporated in the guidelines for the decision-making regarding CABG and PCI, but these thresholds apply to patients with left main and/or three-vessel disease.[19] Our population also consisted of patients with single or two vessel disease and hence, understandably, our mean SXscore and cut-off values for the tertiles were relatively low. It warrants further research to assess which absolute SXscore thresholds are applicable in a heterogeneous population for risk prediction of adverse outcome in patients with CAD.

Furthermore, we argue that combined IVUS-NIRS intracoronary imaging holds promise for more precise detection and quantification of atherosclerotic burden in patients with CAD, and in the future may even be of interest for the prediction of adverse events. However, further research is warranted to assess the application of combined IVUS-NIRS intracoronary imaging for the prediction of adverse events.

Limitations

This cohort, composed of two prospective studies, has broad inclusion criteria which enable the results to be applicable in a broad patient population with CAD. Data collec-

tion, processing and analyses were conducted by researchers independent and blinded for patient and outcome data. However, a few limitations deserve consideration.

As indicated, our study includes patients with relatively low SXscores. This might induce an underestimation of the studied associations and insufficient power to reveal additional associations. However, a subgroup analysis with exclusion of patients without significant CAD, showed results that were essentially similar. Moreover, the lowest tertile in this cohort contains significantly more patient with a previous PCI, which may indicate an underestimation of the severity of CAD caused by a low SXscore derived at study entry.

Furthermore, while the SXscore analyst was blinded for all patient information, occlusions in STEMI patients were scored as occlusions of unknown duration. In the MI SYNTAXscore study, it was suggested to calculate occlusions in STEMI patients post-wiring.[20] However, the MI SYNTAXscore did not show better performance than the original SXscore calculated in STEMI patients.

Lastly, although literature demonstrated that experienced operators produce reasonable SXscores, the modest reproducibility of the SXscore in general has to be acknowledged.[21] However, because of the overall relatively low angiographic burden of disease in our study population, we expected a fair reproducibility of the SXscore in our study. To address the reproducibility of our SXscores, a second experienced operator, blinded for patient characteristics and previously scored SXscores, repeated SXscore analysis in a representative random sample. Cohen's kappa showed to be 0.91, indicating a good interobserver agreement.

Conclusions

In patients with CAD, there is a clear and significant correlation between a higher SXscore and a higher atherosclerotic burden as assessed by RF-IVUS and NIRS in one non-stenotic segment in a single non-culprit coronary artery.

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SUPPLEMENTARY APPENDIX

Methods coronary intravascular ultrasound

Following CAG, IVUS was performed in a proximal non-stenotic (<50% stenosis) segment of at least 40 mm of a non-culprit artery. The order of preference used for selection of the non-culprit vessel was predefined in the study protocol: 1) left anterior descending artery; 2) right coronary artery; 3) left circumflex artery. All IVUS data were obtained 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 pullback speed of 0.5 mm per second. The baseline IVUS images were sent to an independent core laboratory (Cardialysis, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for baseline patient characteristics and clinical outcome. The RF-IVUS analysis was 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). 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. The composition of atherosclerotic plaque was characterised into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core [22]. Three types of high-risk lesions were identified: 1) thin-cap fibroatheroma (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 (MLA) of ≤4.0 mm^2.[4,23]

Methods near-infrared spectroscopy

In a subset of patients, NIRS imaging was performed in the same segment as IVUS. The NIRS system used consists of a 3.2 Fr rapid exchange catheter, a pullback and rotation device and a console (Infraredx, Burlington, MA, USA), approved by the U.S. Food and Drug Administration. Image acquisition was performed by a motorised catheter pullback at a speed of 0.5 mm/s and 240 rpm. The system performed 1,000 chemical measurements per 12.5 mm. Each measurement interrogated 1 to 2 mm^2 of vessel wall from, approximately, 1 mm in depth from the luminal surface towards the adventitia.[4,5]

The NIRS measurements were used to create a chemogram. The fraction of yellow pixels from the chemogram was multiplied by 1,000, to calculate the lipid core burden index (LCBI). Thus, the LCBI value, with a range between 0 and 1,000, represents the amount of lipid core in the assessed segment. [24] In addition, within this region of interest, the 10 mm and 4 mm segment with the highest LCBI was defined. NIRS images

were analysed offline by an independent core laboratory (Cardialysis, Rotterdam, the Netherlands). Core laboratory personnel were blinded to all other patient and outcome data.

Chapter 3

Near-infrared spectroscopy-derived lipid core burden index predicts adverse cardiovascular outcome in patients with coronary artery disease during long-term follow-up

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ABSTRACT

Aims

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

Methods and results

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

Conclusion

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

INTRODUCTION

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

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

METHODS

Study design and population

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

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

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

Near-infrared spectroscopy

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

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

Intravascular ultrasound

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

Study endpoints

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

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

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

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

Statistical analysis

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

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

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

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

RESULTS

Baseline characteristics

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

Incidence of primary endpoint

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

Table 1. Baseline characteristics

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

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

Association between LCBI and MACE

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

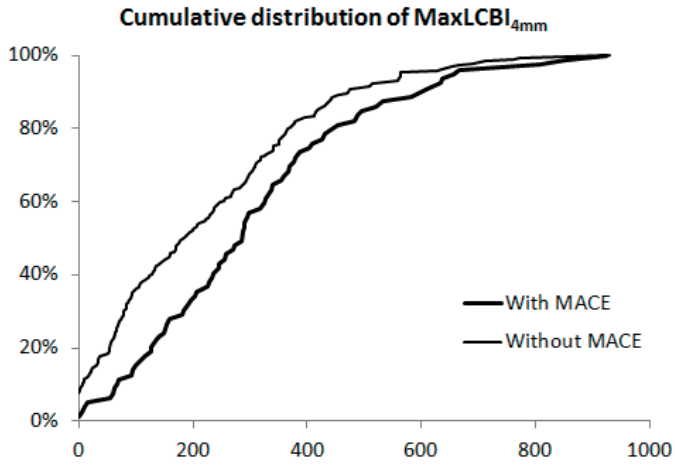
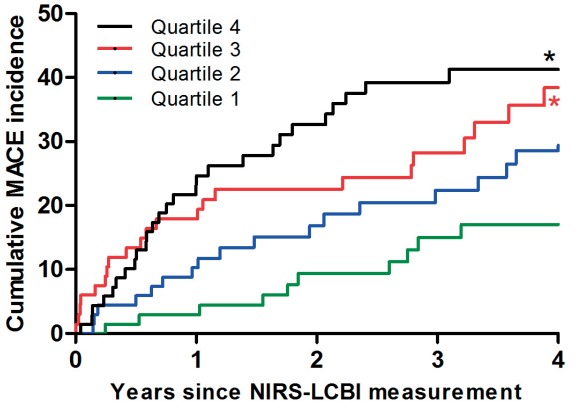


Figure 1. Cumulative distribution of the $MaxLCBI_{4mm}$ of patients with and without MACE (p=0.001, Mann-Whitney U test).
MACE: major adverse cardiac events, $MaxLCBI_{4mm}$: the 4 mm long segment with the maximum LCBI

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

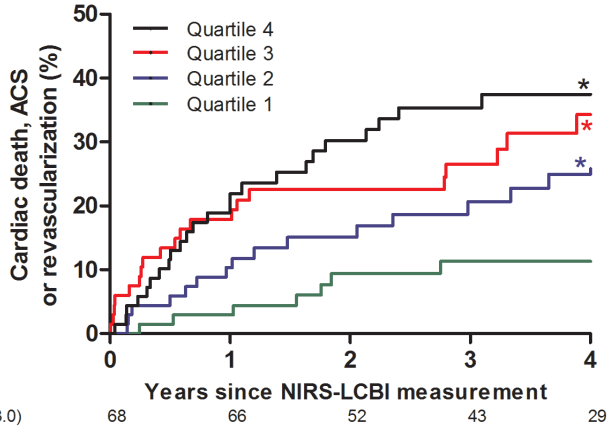
There was a statistically significant continuous relationship between higher $MaxLCBI_{4mm}$ values and a higher risk of MACE (Table 3). After multivariable adjustment, $MaxLCBI_{4mm}$ remained significantly associated with MACE (HR 1.19 per 100 units increase in LCBI, 95%CI: 1.07-1.32), as well as with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

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



No. at risk					
LCBI Q1(<83.0)	68	66	52	43	29
LCBI Q2 (≥83.0-227.0)	68	61	47	40	31
LCBI Q3 (≥227.0-360.0)	67	55	42	35	22
LCBI Q4 (≥360.0)	70	53	41	30	25

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



No. at risk					
LCBI Q1(<83.0)	68	66	52	43	29
LCBI Q2 (≥83.0-227.0)	68	61	47	40	31
LCBI Q3 (≥227.0-360.0)	67	55	42	35	22
LCBI Q4 (≥360.0)	70	53	41	31	26

Figure 3. Assocation between quartiles of MaxLCBI$_{4mm}$ and the occurrence of *cardiac* death, non-fatal ACS and unplanned revascularization
*p<0.05 as compared to first quartile (reference).
LCBI: lipid core burden index, MACE: major adverse cardiac events, NIRS: near-infrared spectroscopy

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

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

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

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

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

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

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

The cumulative incidence of the composite of cardiac death, non-fatal ACS or unplanned revascularization was higher in patients in the second (25.0%), third (31.3%) and fourth (35.7%) quartile of $MaxLCBI_{4mm}$ as compared to those in the first (10.3%) quartile of $MaxLCBI_{4mm}$ (log-rank pairwise comparisons p=0.031, p=0.002 and p=<0.001, respectively, Figure 3). The second, third and fourth quartiles of $MaxLCBI_{4mm}$ were significantly associated with the composite of cardiac death, non-fatal ACS or unplanned revascularization after adjustment for clinical characteristics and IVUS-derived plaque burden in the multivariable model (Table 4). A similar significant association was observed for $MaxLCBI_{4mm}$ as a continuous variable (Table 5). This association persisted after exclusion of target lesion-related events and after exclusion of events related to the imaged segment (Table 5). In general, the associations observed between $MaxLCBI_{4mm}$, $MaxLCBI_{10mm}$ and $LCBI_{ROI}$ and the occurrence of adverse events were stronger and more significant when the composite endpoint included cardiac death instead of all-cause mortality (Tables 2-5).

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

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

Cumulative endpoint incidence by Kaplan-Meier method. P-values obtained with Cox regression analyses on pairwise comparisons between each quartile and first quartile (reference).

ACS: acute coronary syndrome, CI: confidence interval, HR: hazard ratio, LCBI: lipid core burden index, ROI: region of interest

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

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

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

DISCUSSION

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

Studies on the relationship between LCBI and (long-term) follow-up are scarce. Recently, the COLOR study demonstrated that the $MaxLCBI_{4mm}$ obtained prior to stenting in a culprit coronary segment was not associated with culprit-related MACE during 2 years of follow-up.[15] Our study provides new evidence on the prognostic value of NIRS, since we demonstrated that NIRS is predictive of MACE on the long-term by identifying high-risk lipid rich core-containing plaques in a non-culprit artery. The upcoming Lipid Rich Plaque (LRP) and PROSPECT-2 studies are also investigating the ability of NIRS-derived LCBI in non-culprit coronary arteries to predict adverse cardiovascular outcome during 2-year follow-up.

This study extends our previous 1-year follow-up data of the ATHEROREMO-NIRS study, which investigated the 1-year prognostic value of NIRS in that cohort and showed that high LCBI values were associated with an increased incidence of MACE.[10] The cur-

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

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

Other studies used NIRS to investigate the effect of anti-atherosclerotic therapy on the amount of lipid core-containing plaques. The YELLOW study demonstrated that patients with multivessel CAD treated for 6 to 8 weeks with rosuvastatin showed a reduction of lipid core in obstructive arteries.[17] The IBIS-3 study showed that high-dose rosuvastatin resulted in a neutral effect on lipid rich core-containing plaques as determined by NIRS.[18] Recently, it was shown that addition of a PCSK9-inhibitor to stable statin therapy resulted in a greater decrease of plaque burden as assessed by IVUS.[19] NIRS has improved ability to identify lipid core-containing coronary plaques as compared to other invasive imaging modalities including IVUS, since NIRS is able to distinguish cholesterol from other tissue characteristics.[6] In this context, NIRS may be used to select patients with high LCBI values in future research to measure the effect of anti-atherosclerotic therapy on lipid rich core-containing plaques in the coronary artery wall and assess its association with adverse cardiac outcome. Ultimately, this may result in improved risk stratification and management of patients with CAD.

Limitations

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

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

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

Conclusions

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

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Chapter 4

Prognostic value of intravascular ultrasound in patients with coronary artery disease

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ABSTRACT

Background

It has been shown that intravascular ultrasound (IVUS) and radiofrequency (RF-)IVUS can detect high-risk coronary plaque characteristics.

Objectives

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

Methods

From 2008 to 2011, (RF-)IVUS was performed in 1 non-stenotic segment of a non-culprit coronary artery in 581 patients undergoing coronary angiography for acute coronary syndrome (ACS) or stable angina. The pre-defined primary endpoint was major adverse cardiovascular events (MACE), defined as the composite of all-cause death, nonfatal ACS, or unplanned revascularization. Hazard ratios (HRs) were adjusted for age, sex, and clinical risk factors.

Results

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

Conclusions

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

INTRODUCTION

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

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

METHODS

Study design and population

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

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

All patients provided written informed consent which included approval for long-term follow-up. The ATHEROREMO-IVUS study was registered at ClinicalTrialsgov.org (NCT01789411).

Intravascular ultrasound

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

Grayscale IVUS measurements included segmental plaque volume and plaque burden. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Segmental plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. A coronary lesion was defined as a segment with a plaque burden of > 40% in at least 3 consecutive frames. Using RF-IVUS analyses, compositional features of coronary lesions were classified as fibrous, fibro-fatty, necrotic core or dense calcium.[5,15,16] Confluent necrotic core or dense calcium, or the contact of necrotic core with the lumen, were assessed by visual examination performed independently by three investigators blinded to outcome data. Coronary lesions were further classified into 8 different lesion types.[7,15,16] The mentioned criteria should be present in three consecutive frames for a lesion to be considered of a particular category. Three lesions, as identified by (RF-)IVUS, were considered as lesions associated with a high risk for subsequent adverse cardiac events; 1) TCFA lesion, defined as a lesion with the presence of >10% confluent necrotic core in direct contact with the lumen; 2) lesion with a plaque burden ≥70%; 3) lesion with a minimal luminal area ≤4.0mm^2.[15]

Follow-up

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

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

Study endpoints

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

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

Based on original source data of available coronary angiography and hospital records at the time of the event, the clinical event committee adjudicated (blinded to IVUS data) whether the event was related to the coronary site that had been treated during the index procedure (culprit lesion–related event) or as related to a coronary site that had not been treated during the index procedure (non-culprit lesion–related event). Events that were related to both the culprit lesion and a non-culprit site (e.g., revascularization of multiple vessels with coronary artery bypass grafting) were classified into both categories. When information was not sufficient to classify an event as either culprit lesion related or non-culprit lesion related, the event was classified as indeterminate.[15]

Statistical analysis

Normally distributed continuous variables were reported as mean ± SD. Nonnormally-distributed variables were reported as median (interquartile range [IQR]). Categorical variables were reported as numbers and percentages.

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

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

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

All statistical tests were 2-tailed, and p values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS statistics version 21.0 (IBM Corp., Armonk, New York).

RESULTS

Baseline characteristics

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

Table 1. Baseline characteristics

	N=581 patients
Clinical characteristics	
Age, years	61.6 ± 11.3
Men, n(%)	439 (75.6)
Diabetes Mellitus, n(%)	99 (17.0)
Hypertension, n(%)	300 (51.6)
Dyslipidemia, n(%)	321 (55.2)
Current smoking, n(%)	169 (29.1)
Positive family history, n(%)	301 (51.8)
Previous MI, n(%)	184 (31.7)
Previous PCI, n(%)	186 (32.0)
Previous CABG, n(%)	18 (3.1)
Previous CVA, n(%)	26 (4.5)
History of peripheral artery disease, n(%)	36 (6.2)
History of renal impairment, n(%)	32 (5.5)
History of heart failure, n(%)	19 (3.3)
Median C-reactive protein, mg/L	2.1 [0.9-5.4]
Procedural characteristics	
Indication for coronary angiography	
Acute MI, n(%)	167 (28.7)
Unstable angina, n(%)	151 (26.0)
Stable angina, n(%)	254 (43.7)
Other, n(%)	9 (1.5)
PCI performed, n(%)	511 (88.0)
Coronary artery disease	
No significant stenosis, n(%)	43 (7.4)
1-vessel disease, n(%)	308 (53.0)
2-vessel disease, n(%)	168 (28.9)
3-vessel disease, n(%)	62 (10.7)
IVUS characteristics	
Imaged coronary artery	
Left anterior descending, n(%)	210 (36.1)
Left circumflex, n(%)	195 (33.6)
Right coronary artery, n(%)	176 (30.3)
Median imaged segment length, mm	44.3 [33.8-55.4]
Median segmental plaque burden, %	39.1 [30.0-46.4]
Median segmental plaque volume, mm^3	222.7 [136.1-326.6]

CABG: coronary artery bypass graft, CVA: cerebrovascular accident, IVUS: intravascular ultrasound, MI: myocardial infarction, PCI: percutaneous coronary intervention

Incidence of study endpoints

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

Table 2. Incidence of composite endpoints

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

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

Association between (RF-)IVUS and MACE

The presence of a lesion with a minimal luminal area ≤4.0 mm2 was significantly and independently associated with MACE (cumulative MACE incidence when present: 33.9% vs. 22.2% when absent; adjusted HR: 1.49; 95% CI: 1.07 to 2.08; p = 0.020) (Table 3). Furthermore, the presence of a TCFA lesion with a plaque burden ≥70% was significantly associated with MACE (cumulative MACE incidence when present: 37.7% vs. 24.6% when absent; adjusted HR: 1.73; 95% CI: 1.12 to 2.66; p = 0.013), while the presence of a TCFA lesion or a lesion with a plaque burden ≥70% itself was not independently associated with MACE (Table 3). After multivariable adjustment, segmental plaque burden and plaque volume remained no longer independently associated with MACE (Table 3). The results were essentially similar when definite culprit lesion–related events were excluded. Cox regression analysis with follow-up duration as a time-dependent variable showed that both the presence of a TCFA lesion and a lesion with a plaque burden ≥70% were strong predictors of MACE for the first year of follow-up, but not beyond

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

	Unadjusted model		Full model	
	HR (95% CI)	p-value	HR (95% CI)	p-value
MACE				
TCFA	1.20 (0.87-1.65)	0.27	1.27 (0.91-1.77)	0.16
PB ≥70%	1.50 (1.05-2.16)	0.028	1.33 (0.92-1.93)	0.13
MLA ≤4.0 mm^2	1.57 (1.13-2.17)	0.007	1.49 (1.07-2.08)	0.020
TCFA + PB ≥70%	1.90 (1.25-2.90)	0.003	1.73 (1.12-2.66)	0.013
TCFA + MLA ≤4.0 mm^2	1.47 (0.93-2.33)	0.10	1.50 (0.93-2.44)	0.10
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.64 (0.93-2.89)	0.089	1.74 (0.97-3.13)	0.066
PB≥70% + MLA ≤4.0 mm^2	1.29 (0.83-2.01)	0.26	1.30 (0.82-2.04)	0.26
Segmental plaque burden, %	1.24 (1.07-1.44)	0.004	1.15 (0.98-1.34)	0.079
Segmental plaque volume, mm^3	1.07 (0.96-1.20)	0.23	1.02 (0.90-1.14)	0.79
Composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization				
TCFA	1.04 (0.73-1.49)	0.83	1.12 (0.77-1.61)	0.56
PB ≥70%	1.63 (1.10-2.42)	0.014	1.43 (0.96-2.15)	0.083
MLA ≤4.0 mm^2	1.85 (1.30-2.64)	0.001	1.82 (1.26-2.64)	0.001
TCFA + PB ≥70%	1.95 (1.23-3.09)	0.005	1.78 (1.11-2.85)	0.017
TCFA + MLA ≤4.0 mm^2	1.74 (1.08-2.81)	0.023	1.86 (1.11-3.10)	0.018
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.84 (1.02-3.34)	0.044	2.09 (1.12-3.89)	0.020
PB≥70% + MLA ≤4.0 mm^2	1.45 (0.91-2.32)	0.12	1.51 (0.93-2.45)	0.093
Segmental plaque burden, %	1.28 (1.09-1.50)	0.003	1.17 (0.99-1.39)	0.070
Segmental plaque volume, mm3	1.06 (0.93-1.20)	0.40	0.98 (0.86-1.12)	0.80
Composite endpoint of cardiac death, non-fatal ACS or unplanned revascularization exclusive of culprit lesion-related events				
TCFA	0.99 (0.66-1.48)	0.95	1.07 (0.70-1.62)	0.76
PB ≥70%	2.08 (1.36-3.18)	0.001	1.66 (1.06-2.58)	0.026
MLA ≤4.0 mm2	2.03 (1.37-3.03)	<0.001	1.88 (1.24-2.83)	0.003
TCFA + PB ≥70%	2.17 (1.32-3.59)	0.002	1.84 (1.10-3.07)	0.021
TCFA + MLA ≤4.0 mm^2	1.64 (0.95-2.84)	0.078	1.75 (0.98-3.13)	0.059
TCFA + PB ≥70% + MLA ≤4.0 mm^2	1.97 (1.02-3.79)	0.042	2.03 (1.03-4.02)	0.041
PB≥70% + MLA ≤4.0 mm^2	1.77 (1.07-2.92)	0.026	1.73 (1.04-2.90)	0.035
Segmental plaque burden, %	1.41 (1.18-1.69)	<0.001	1.26 (1.03-1.52)	0.022
Segmental plaque volume, mm^3	1.13 (0.99-1.30)	0.080	1.03 (0.89-1.20)	0.68

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

1-year follow-up. On the contrary, a lesion with a minimal luminal area ≤4.0 mm2 itself was not an independent predictor in the first year of follow-up (adjusted HR: 1.40; 95% CI: 0.83 to 2.34; p = 0.21), but did predict MACE beyond 1-year of follow-up (1-year to 5-year follow-up adjusted HR: 1.58; 95% CI: 1.04 to 2.40; p = 0.032). Results remained essentially similar when we performed an exploratory multivariable analysis applying the model used for the 1-year follow-up data (which comprised 6 variables instead of the 11 variables used in the model for the current analyses) (Table 4).

Table 4. Associations of (RF-)IVUS characteristics and risk of MACE at 4.7-years of follow-up using two different models for multivariable adjustment

	1-year follow-up data adjusted model with 6 covariates		5-year follow-up data adjusted model with 6 covariates		5-year follow-up data adjusted model with 11 covariates	
	HR (95% CI)	p-value	HR (95% CI)*	p-value	HR (95% CI)†	p-value
MACE						
TCFA	1.98 (1.09-3.60)	0.026	1.25 (0.90-1.73)	0.18	1.27 (0.91-1.77)	0.16
PB≥70%	2.90 (1.60-5.25)	<0.001	1.42 (0.99-2.05)	0.059	1.33 (0.92-1.93)	0.13
MLA≤4.0mm2	1.23 (0.67-2.26)	0.50	1.51 (1.09-2.09)	0.014	1.49 (1.07-2.08)	0.020
TCFA+PB≥70%	-	-	1.80 (1.18-2.75)	0.007	1.73 (1.12-2.66)	0.013
TCFA+ MLA≤4.0mm2	-	-	1.61 (1.01-2.57)	0.046	1.50 (0.93-2.44)	0.10
TCFA+PB≥70%+ MLA≤4.0mm2	-	-	1.82 (1.02-3.26)	0.043	1.74 (0.97-3.13)	0.066
Segmental plaque burden (per 10 units increase)	-	-	1.17 (1.01-1.36)	0.040	1.15 (0.98-1.34)	0.079
Segmental plaque volume (per 100 units increase)	-	-	1.03 (0.92-1.16)	0.63	1.02 (0.90-1.14)	0.79

* Variables entered in the 6-covariate model were age, sex, diabetes mellitus, hypertension, history of percutaneous coronary intervention, and indication for coronary angiography.
† Variables entered in the 11-covariate model were age, sex, diabetes mellitus, hypertension, history of percutaneous coronary intervention, indication for coronary angiography, dyslipidemia, history of myocardial infarction, history of coronary artery bypass grafting, history of peripheral artery disease, and percutaneous coronary intervention performed at index.
CI: confidence interval, HR: hazard ratio, IVUS: intravascular ultrasound, MACE: major adverse cardiovascular events, MLA: minimal luminal area, PB: plaque burden, RF: radiofrequency, TCFA: thin-cap fibroatheroma

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

The presence of a lesion with a minimal luminal area ≤4.0 mm2 was also significantly and independently associated with a higher rate of the composite endpoint of cardiac death, nonfatal ACS, or unplanned revascularization (cumulative incidence of composite endpoint when present: 30.6% vs. 16.9% when absent; adjusted HR: 1.82; 95% CI: 1.26 to 2.64; p = 0.001) (Figure 1, Table 3). The same was true for TCFA lesions with a plaque burden ≥70% or a minimal luminal area ≤4.0 mm2 (Table 3). The highest risk, in

terms of adjusted HRs, was among patients who had a TCFA lesion with both a plaque burden ≥70% and a minimal luminal area ≤4.0 mm2 (cumulative incidence of composite endpoint when present: 34.3% vs. 20.7% when absent; adjusted HR: 2.09; 95% CI: 1.12 to 3.89; p = 0.020) (Table 3).

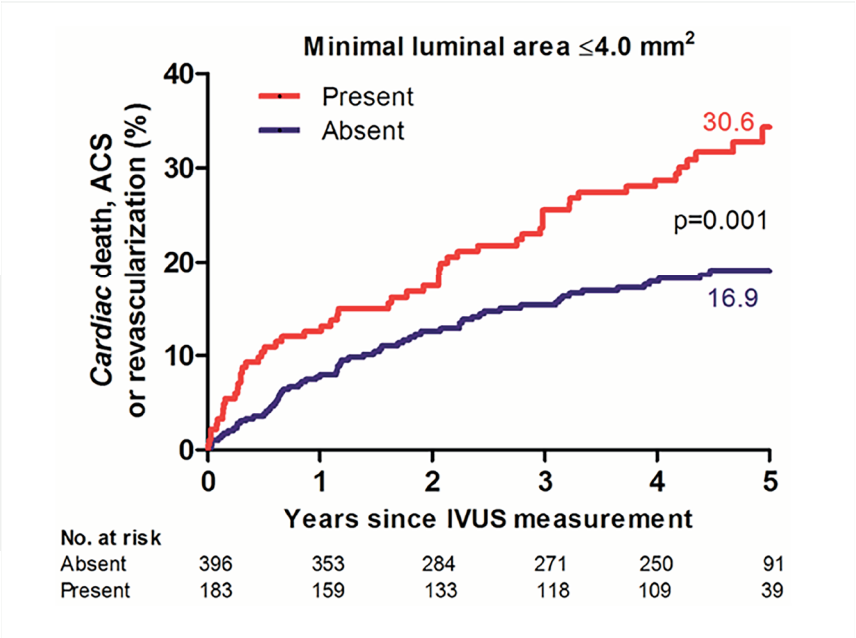

Figure 1. Association between the absence or presence of a lesion with a minimal luminal area ≤4.0 mm^2 and the composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization.

P-value obtained by the Log-rank test.

ACS: acute coronary syndrome, IVUS: intravascular ultrasound

These associations remained essentially unchanged after exclusion of culprit lesion–related events (Figure 2, Table 3). In addition, a significant association was observed for the presence of a lesion with a plaque burden ≥70%, or its combination with a minimal luminal area ≤4.0 mm2, as well as for segmental plaque burden with each 10-U increase in segmental plaque burden resulting in a 26% increase in risk for occurrence of the composite endpoint of cardiac death, nonfatal ACS, or unplanned revascularization after exclusion of culprit lesion-related events (adjusted HR: 1.26 per 10-U increase; 95% CI: 1.03 to 1.52; p =0.022) (Figure 2, Table 3).

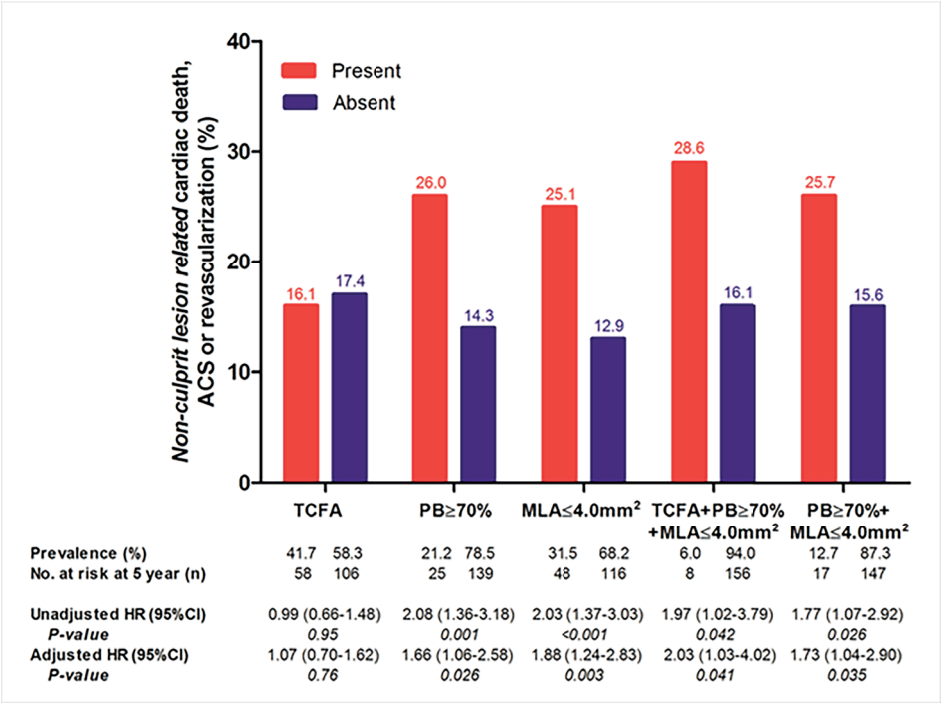

Figure 2. Association between (RF-)IVUS derived lesion characteristics and the composite endpoint of *cardiac* death, non-fatal ACS or unplanned revascularization, after exclusion of culprit lesion-related events.

Percentages are cumulative events rates estimated by the Kaplan-Meier method. Prevalence (%) in the footer refers to the prevalence of the specific (RF-)IVUS characteristic. P-values are obtained by the Log-rank test. Hazard ratios are estimated by univariate Cox regression analyses.

ACS: acute coronary syndrome, HR: hazard ratio, MLA: minimal luminal area, PB: plaque burden, TCFA: thin-cap fibroatheroma

DISCUSSION

This 4.7-year follow-up of the ATHEROREMO-IVUS study demonstrated that a small luminal area and a large plaque burden, but not RF-IVUS–derived compositional plaque features on their own, are independent determinants of (nonculprit lesion–related) adverse cardiac events in patients with CAD. The increased risk associated with a minimal luminal area ≤4.0 mm2 was not observed at 1-year follow-up,[15] whereas the prognostic value of plaque burden ≥70% was confirmed, although statistical significance was not consistently present for all different composite endpoints. In contrast, the independent association between a TCFA lesion as an isolated characteristic and adverse outcome at 1 year did not persist during long-term follow-up. Still, patients with a TCFA lesion with a large plaque burden and/or a small luminal area were at increased risk.

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

We consider several possible explanations for the inconsistent association between the presence of a TCFA lesion as an isolated characteristic and the risk of adverse cardiac events during short-term versus long-term follow-up. First, controversy exists about the ability of RF-IVUS to correctly discern and identify the thin-cap and necrotic core as individual components of a TCFA lesion, due to the limitations with respect to spatial resolution.[4,19] Secondly, the dynamic nature of TCFA lesions over time should be appreciated, because it has been described that particularly (proximal) TCFA lesions with a large plaque burden heal less often and might have a greater tendency to rupture.[20] This may explain our finding that the presence of a TCFA lesion with a large plaque burden was associated with an increased risk for adverse cardiac events over 4.7-years of follow-up, whereas a TCFA lesion in itself was not. Thirdly, previous studies have demonstrated that a lesion with a large plaque burden is a consistent and prevalent predictor for adverse cardiac outcome. However, whereas the atherosclerotic disease burden has been shown as a consistent and strong predictor of adverse cardiovascular events, no study has yet demonstrated that a TCFA lesion by itself independently predicts adverse cardiovascular outcome after adjustment for plaque burden and other potential confounders.[13,14,21,22]

Our current study suggests that a RF-IVUS-derived TCFA lesion only has long-term prognostic value if accompanied with other high-risk plaque features. Therefore, this study further adds to the discussion as to whether RF-IVUS offers incremental prognostic value to grayscale IVUS in terms of identification of high-risk coronary plaque phenotypes based on compositional features. In addition, our current study demonstrates for the first time that (RF-)IVUS plaque characteristics, as assessed in one non-stenotic segment of a non-culprit coronary artery, predicts adverse cardiovascular events in patients with CAD during long-term follow-up. A post hoc analysis did not show heterogeneity in the HR estimates in patients with ACS versus SAP. Moreover,

the large number of endpoints allowed for a separate analysis with exclusion of culprit-lesion related endpoint events, with results that remained essentially unchanged. This indicates that (RF-)IVUS-derived plaque characteristics, as identified in one non-culprit coronary artery segment, may reflect atherosclerotic vulnerability of the entire coronary tree.

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

Study limitations

First, the number of TCFA lesions might be overestimated by RF-IVUS because of the limited spatial resolution with respect to the identification of the thin cap of a TCFA lesion. Second, IVUS imaging was not repeated during follow-up. Therefore, we could not account for the potential dynamic nature of coronary lesions. It should also be noted that this study does not provide insight into how the individual lesion correlates to the adverse event. Third, the follow-up questionnaire was completed by 86% of the patients. Although, for the majority of the remaining patients, follow-up information was retrieved from our local hospital records, we cannot fully exclude the possibility that loss to follow-up was in part selective. However, our study reflects daily clinical practice, because patients admitted with both ACS and SAP were included. Besides, the current study represents a long-term study investigating the association between (RF-)IVUS-derived plaque characteristics and adverse cardiovascular outcome during 4.7-years of follow-up in patients with ACS or SAP, which represents the longest follow-up reported so far.

Conclusions

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

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Chapter 5

SYNTAX score II predicts long-term mortality in patients with one- or two-vessel disease

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ABSTRACT

Objective

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

Methods

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

Results

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

Conclusion

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

INTRODUCTION

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

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

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

METHODS

Study design and population

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

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

SYNTAX score II

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

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

Study endpoint

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

Statistical analysis

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

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

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

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

RESULTS

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

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

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

Table 1. Baseline characteristics

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

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

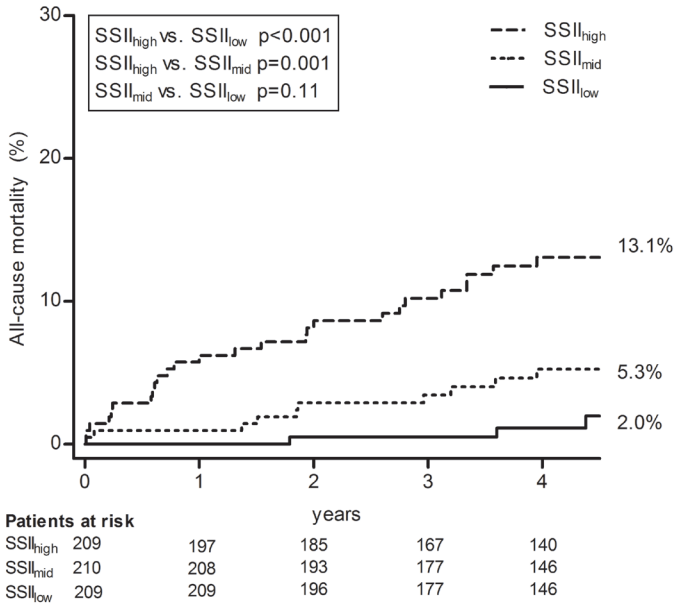

Figure 1. Cumulative incidence of all-cause mortality at 4.5 years

SSII is divided in tertiles with cut-off points 17 and 24 to compare the cumulative all-cause mortality proportions between patients with a low, mid or high SSII value.

SSII: Syntax score II

Table 2. Prediction of long-term mortality

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

SSII incorporates the anatomical Syntax score, age, gender, creatinine clearance, left ventricular ejection fraction, peripheral vascular disease and chronic obstructive pulmonary disease.

CAG: coronary angiography, CI: confidence interval, HR: hazard ratio, PCI: percutaneous coronary intervention, SAP: stable angina pectoris, SSII: Syntax score II

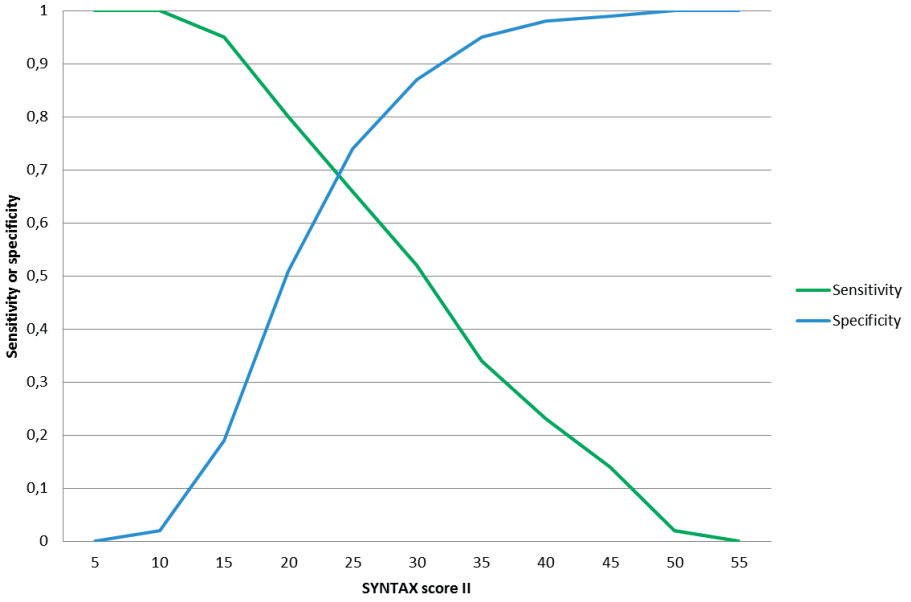

Figure 2. Sensitivity and specificity of SSII for the long-term prediction of all-cause mortality

DISCUSSION

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

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

that other clinical variables incorporated in the SSII, such as CRCL, sufficiently reflect the influence of diabetes mellitus. In this respect, a previous study has demonstrated that kidney disease is of greater importance than diabetes mellitus for risk prediction of adverse outcome in patients with CAD.[14]

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

Limitations

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

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

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

Conclusion

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

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Chapter 6

Temporal evolution of Myeloperoxidase and Galectin 3 during 1 year after acute coronary syndrome admission

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ABSTRACT

Prior studies reported that Myeloperoxidase and Galectin-3, which are biomarkers of coronary plaque vulnerability, are elevated in acute coronary syndrome (ACS) patients. We studied the temporal evolution of these biomarkers early after ACS admission and prior to a recurrent ACS event during 1 year follow-up.

INTRODUCTION

Myeloperoxidase (MPO) and Galetin-3 (GAL-3) are pro-inflammatory proteins that promote plaque vulnerability through various mechanisms such as nitric oxide catalysation, foam cell formation and vascular smooth muscle cell dedifferentiation.[1, 2] Both biomarkers, measured at admission, have been associated with cardiac death and non-fatal myocardial infarction (MI) during follow-up in patients with acute coronary syndrome (ACS).[1, 3, 4] Since plaque vulnerability and thus coronary artery disease (CAD) is a highly dynamic process, repeated measurements of MPO and GAL-3 during follow-up may contain additional predictive value in post-ACS patients. To evaluate this hypothesis, we studied the evolution of these biomarkers in detail by means of highly frequent serial measurements during one year after ACS admission.

METHODS

Study Design

The BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS) was designed to reveal temporal evolutions of cardiovascular (CV) biomarkers during 1 year follow-up in ACS patients.[5, 6] Differences in temporal changes between patients with and without a recurrent ACS (reACS) were of particular interest. A total of 844 patients were enrolled in 18 Dutch hospitals, who were aged ≥40 years and had ≥1 CV risk factor. Blood sampling was scheduled every two weeks during the first half-year and monthly during the second half-year, with the first sample taken at admission or at the first outpatient visit (4-6 weeks) after discharge. The study endpoint was defined as the composite of cardiac death, MI, or unstable angina requiring urgent coronary revascularization, and was reached by 45 patients. BIOMArCS was approved by the Institutional Review Boards of all participating hospitals, and all patients gave informed consent. BIOMArCS is registered in The Netherlands Trial Register as NTR1698.

Case-cohort approach

A case-cohort approach was used for biomarker determination and analysis of the temporal evolution during 1-year follow-up.[7] A case-cohort comprises a random sub cohort from the full cohort, together with all patients who reach the study endpoint (‘cases’). It is an efficient analysis method, while study validity and statistical power are maintained.[8] We selected a random sub cohort of 150 patients, which appeared to include 8 cases. Hence, our case-cohort consisted of (all) 45 study endpoint cases and 142 event-free patients. A median of 8 (interquartile range [IQR] 5-11) repeated samples were analyzed per patient, totaling 1478 measurements.

In order to obtain detailed information on biomarker changes early after ACS admission, by design, a series of 68 BIOMArCS patients underwent additional blood sampling at day 1 to 4. We included these patients in an analysis of post-ACS biomarker stabilization, excluding all 45 study endpoint cases to avoid distortion of the biomarkers patterns. As 19 (out of the 68) patients were also part of the case-cohort, a total of 191 patients contributed to a median of 8 (IQR 5-10) repeated samples per patient totaling 1507 measurements for this analysis.[7]

MPO and Gal-3 measurements

Blood samples were collected on-site and frozen at -80℃ within 82 (25th-75th percentile 58-117) minutes after withdrawal. Subsequently, samples were securely transported to the Erasmus MC for long-term storage. Serum samples were used to measure MPO and GAL-3 and quantified batch-wise, blinded for patient characteristics. MPO was measured with a 384-ELISA plate (Nunc, Thermo #460372), with a lower limit of detection of 609 pg/ml. The corresponding 10% coefficient of variation was 5.7%. GAL-3 was measured with a custom built Luminex immune-assay validated in the University Medical Centre Utrecht, the Netherlands. The corresponding lower limit of quantification was 0.06 pg/ml, the upper limit of quantification was 1000 pg/ml and the reference sample value was 158.43 pg/ml. The inter-assay coefficient of variation of the used GAL-3 custom build assay was 13.9% and the intra-assay coefficient of variation was 14.45%.

Statistical analysis

MPO and GAL-3 had skewed distributions, and were log-transformed for analysis purposes. Results are presented on the linear scale.

Linear mixed-effect models (LME) were applied to describe the patterns of MPO and GAL-3 early after the index-ACS. We placed two splines to account for possible non-linearity. Using LME, we calculated the average biomarker values for each post-ACS day. We concluded biomarker stabilization when the (relative) difference in biomarker level between two consecutive days appeared less than one percent.

Joint models, combining LME and Cox proportional hazard regression models, were applied to study the temporal biomarker trajectories in relation to reACS.[7] We included time from index-ACS as main determinant, while adjusting for GRACE risk score, gender, history of diabetes, coronary artery bypass graft, valvular heart disease and peripheral vessel disease. In the Cox model, GRACE risk score was added as potential confounder of the relation between biomarker level and the time-to-event. Additionally, we performed a post-hoc sensitivity analysis using only the data available after biomarker level stabilization to investigate if findings are influenced by early post-ACS elevations and variations in biomarker level.

Results of the joint models are presented as hazard ratios (HR) with corresponding 95% confidence interval (CI) per standard deviation (SD) increase of the biomarker (on the log-scale). All relevant model assumptions were evaluated, including residual plots, and no meaningful deviations were observed.

Analyses were performed with R Statistical Software using packages *nlme* and *JMbayes*. All statistical tests were two-tailed and the α-level of 0.05 was applied to conclude statistical significance.

RESULTS

Median age was 63.6 (25th-75th percentile 55.3-71.6) years, 79.0% were men and index-ACS was classified as STEMI in 43.3% (Table 1). Cases had higher prevalence of diabetes and a higher GRACE risk score than event-free patients.

Table 1. Baseline characteristics

	Endpoint cases	Endpoint-free patients	p-value
Number of patients	45	142	
Age, yr (IQR)	67.4 (57.1-76.5)	62.6 (55.0-70.9)	0.075
Man (%)	36 (80.0)	111 (78.2)	0.79
Cardiovascular risk factors (%)			
Diabetes Mellitus	17 (37.8)	24 (16.9)	0.003
Hypertension	22 (48.9)	77 (54.2)	0.53
Hypercholesterolemia	20 (44.4)	72 (50.7)	0.46
Current smoker	17 (37.8)	60 (42.2)	0.52
Presentation on admission			
Diagnosis			
STEMI	16 (35.6)	65 (45.8)	0.46
NSTEMI	22 (48.9)	56 (39.4)	
Unstable angina pectoris	7 (15.6)	21 (14.8)	
PCI performed	34 (87.2)	109 (82.6)	0.50
GRACE risk score (IQR)	121 (98-141)	109 (88-130)	0.022

Continuous variables are presented as median with IQR. Categorical variables are presented as numbers and percentages.
GRACE risk score: Global Registry of Acute Coronary Events risk score, NSTEMI: non-STEMI, PCI: Percutaneous coronary intervention, STEMI: ST-elevation myocardial infarction, yr: year

Myeloperoxidase

MPO level was elevated early after the index-ACS, with a peak value of 78.0 ng/ml at the day of admission. Within the first seven days, MPO showed a steep decline, and then stabilized after day 6 at 25.7 ng/ml (Figure 1A). During follow-up, MPO levels in cases and event-free patients were similar: after seven days, the average serum level

of MPO was 26.4 (IQR: 22.1-32.4) ng/ml in cases and 25.3 (IQR: 19.9-31.9) ng/ml in endpoint-free patients. We did not observe a steady or sudden increase in MPO level prior to the reACS event (Figure 1B). The unadjusted HR for reACS per SD increase in MPO was 0.84 (95% CI 0.61-1.26) Adjustment for multiple factors did not result in a meaningful change of the estimate (Table 2).

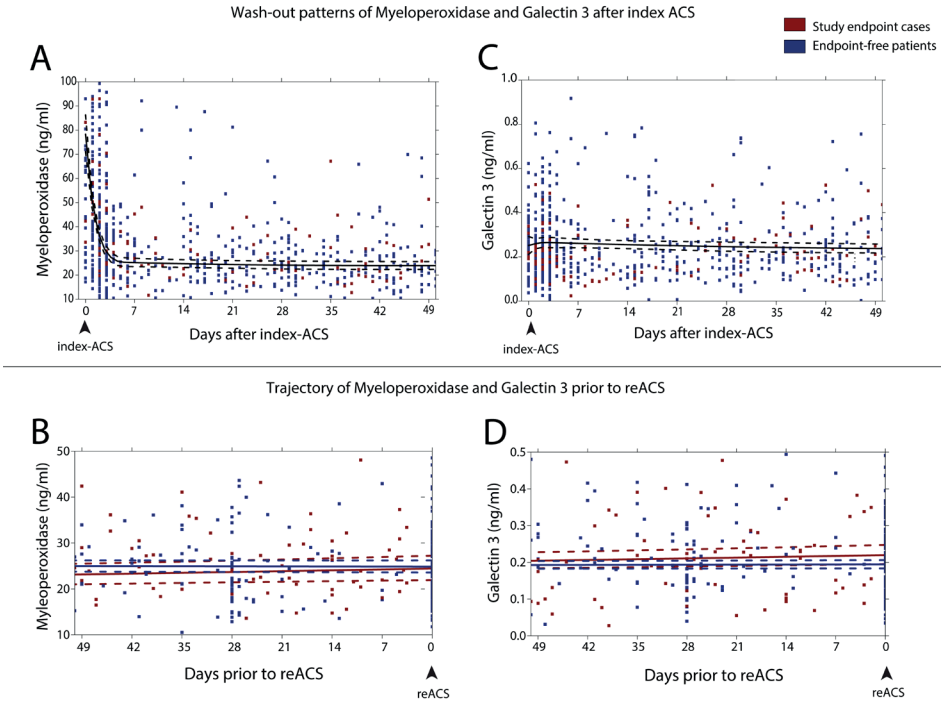

Figure 1. Temporal evolution of Myeloperoxidase and Galectin 3

Panel 1A and 1C depict the early time-course of MPO and GAL-3 after the index-ACS. Panel 1B and 1D depict the median value of the patient-level mean of MPO and GAL-3 prior to reACS in study endpoint cases and endpoint-free patients.

Galectin-3

Gal-3 was only slightly elevated at the index-ACS, and stabilized after day 3 at 0.21 ng/ml (Figure 1C). Gal-3 remained constant during follow-up, and mean levels did not differ between cases and event-free patients (Figure 2D). After 7 days, the average serum level of GAL-3 was 0.24 (IQR: 0.16 – 0.30) ng/ml in cases and 0.23 (IQR: 0.17 – 0.30) ng/ml in endpoint-free patients. Prior to reACS, we observed no steady or sudden elevation in GAL-3 in cases. The unadjusted HR for reACS per SD increase in GAL-3 was 1.41 (95% CI 0.77-2.42), which remained unaltered after multiple adjustment (Table 2).

Table 2. Associations between biomarker trajectories and study endpoints

	- 1 SD	Mean	+ 1 SD	unadjusted HR	p-value	Adjusted HR*	p-value
Myeloperoxidase, ng/ml†	15.6	26.2	44.0	0.84 (0.61 - 1.26)	0.32	0.84 (0.56 - 1. 37)	0.44
Galectin 3, ng/ml†	0.10	0.20	0.38	1.41 (0.77 - 2.42)	0.27	1.56 (0.87 - 2.53)	0.12

Sensitivity analysis with post 7 days data

	- 1 SD	Mean	+ 1 SD	unadjusted HR	p-value	Adjusted HR*	p-value
Myeloperoxidase, ng/ml‡	15.7	24.7	38.8	1.00 (0.62 - 1.69)	1.00	1.18 (0.74 – 1.91)	0.52
Galectin 3, ng/ml‡	0.10	0.20	0.38	1.33 (0.63 - 3.09)	0.47	1.02 (0.48 - 2.15)	0.95

† based on 1478 measurements in 187 patients (median: 8 [IQR: 5-11])
‡ based on 1282 measurements in 174 patients (median: 8 [IQR: 4-10])
*Cox model adjusted for GRACE risk score, mixed model adjusted for GRACE risk score; gender; history of diabetes, coronary artery bypass graft, valvular heart disease, peripheral vessel disease
HR: hazard ratio, ml: microliter, ng: nanogram, SD: standard deviation

DISCUSSION

We established the detailed temporal trajectories of MPO and GAL-3 in post-ACS patients by means of frequently serial measurements. MPO was elevated at the time of the index-ACS, and decreased and stabilized within 7 days. longitudinal MPO levels were not associated with reACS. In particular, no increase in MPO level was observed prior to a recurrent event. Similar results were observed with respect to GAL-3: there were no differences in longitudinal evolution between reACS cases and event-free patients.

MPO is a pro-inflammatory biomarker involved in multiple inflammatory processes that propagate plaque instability, such as nitric oxide catalysation, leukocyte attraction, endothelial cell apoptosis and tissue factor activation stimulating thrombosis.[2] GAL-3 is also reckoned a pro-inflammatory biomarker stimulating plaque instability by i.e. monocyte attraction, macrophage polarization, foam cell production and vascular smooth muscle cell dedifferentiation [1]. Because of their inflammatory character, MPO and GAL-3 may destabilize plaques susceptible to thrombosis, leading to reACS in post-ACS patients.[1, 9] A recent meta-analysis showed that higher MPO levels measured at baseline, are associated with adverse outcome.[9] As for GAL-3, opposite results have been found regarding its prognostic value in post-ACS patients.[10-12]

BIOMArCS was specifically designed to study the temporal evolution of serum biomarkers in post-ACS patients, and its highly frequent blood sampling schedule would have sufficed to identify meaningful changes in MPO and GAL-3 concentrations, had they appeared. However, contrary to our expectations, both biomarkers were not associated with an increased risk of a recurrent ischemic event during 1-year follow-up. Since the median time between the last collected sample in cases and their reACS was 11 (IQR: 5-20) days, we cannot exclude that just before reACS there might still have been biomarker elevations we did not detect. Additionally, we cannot exclude changes

in MPO or GAL-3 levels during long-term follow-up. Nonetheless, it seems that MPO and GAL-3 do not advance plaque vulnerability prior to reACS.

Conclusion

MPO and to a lesser extend GAL-3 were elevated early after, but not before a clinical symptomatic ACS. Post-ACS patients who experienced a recurrent event within one year were not characterized by elevated levels of these pro-inflammatory biomarkers. Also steady or sudden elevations were absent, hence MPO and GAL-3 appear unsuited for prognosis monitoring after ACS.

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Chapter 7

Temporal pattern of Growth Differentiation Factor-15 (GDF-15) protein after acute coronary syndrome: results of the BIOMArCS study

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ABSTRACT

Growth differentiation factor-15 (GDF-15) has appeared as a promising biomarker with strong predictive abilities in acute coronary syndrome (ACS). However, studies are solely based on *single* measurements in the *acute* phase of an ACS event. The way GDF-15 patterns in post ACS patients behave on the long term is largely unknown. We conducted a nested case-control study within our multicenter, prospective, observational biomarker study (BIOMArCS) of 844 ACS patients. Following an index ACS event, high-frequency blood sampling was performed during 1-year of follow-up. GDF-15 was determined batchwise by electrochemiluminescence immunoessays in 37 cases with a recurrent event during 1-year follow-up, and in 74 event-free controls. Cases and controls had a mean ± standard deviation age of 66.9 ± 11.3 years and 81% were men. From 30 days onwards, patients showed stable levels, which were on average 333 (95% confidence interval 68-647) pg/mL higher in cases than controls (1704 vs. 1371 pg/mL; p-value 0.013). Additionally, in the post 30-day period, GDF-15 showed low within-individual variability in both cases and controls. In conclusion, post ACS patients experiencing a recurrent event had stable and systematically higher GDF-15 levels during 30-day to 1-year follow-up than their event-free counterparts with otherwise similar clinical characteristics. Thus, post-discharge blood sampling might be used throughout the course of 1 year to improve prognostication, whereas, in view of the low within-individual variation, the number of repeated sampling moments might be limited.

INTRODUCTION

In recent years, circulating growth differentiation factor-15 (GDF-15), a stress-induced cytokine, has emerged as a biomarker of interest due to its potential prognostic value in patients with cardiovascular disease.[1] In particular, elevated levels of GDF-15 are associated with an impaired prognosis after acute coronary syndromes (ACS).[2-6] However, the prognostic value of GDF-15 in ACS patients thus far, has been mainly based on *single* measurements in the *early*, *acute* phase of an acute ischemic event. Therefore, the optimal time point in the stabilized post ACS phase for GDF-15 blood sampling to make prognostic implications remains not fully elucidated yet. We used our 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) with high-frequency blood sampling in post ACS patients as a platform to describe the temporal evolution of GDF-15 during 1-year follow-up, to evaluate differences between patients with and without a recurrent event, and to study the individual variability of GDF-15.

METHODS

We performed a nested case-control analysis within the main BIOMArCS study that was approved by the medical ethics committees of all participating hospitals. The rationale and design of BIOMArCS are described in detail elsewhere.[7] In brief, BIOMArCS is a prospective, multicenter, observational study conducted in 18 participating hospitals in the Netherlands. A total of 844 patients, admitted for an ACS, including unstable angina pectoris, non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) and with at least one additional cardiovascular risk factor, were enrolled between 2008 and 2015. Patients underwent regular blood sampling after the initial admission for ACS according to a strict schedule to describe the temporal evolution of blood biomarkers in the post ACS phase, and to reveal deviations in temporal biomarker patterns prior to a recurrent coronary event. Venepuncture was performed at admission, at hospital discharge and subsequently every fortnight during the first half year, followed by monthly blood sample collection until 1 year. Follow-up blood sampling was terminated permanently after coronary artery bypass grafting, hospital admission for heart failure, or a detoriation of renal function leading to a glomerular filtration rate of < 30 mL/min/1.73m^2, since these conditions influence circulating biomarker concentrations. Ultimately, patients had 17 (median) repeated blood samples within 1 year. The study was performed in accordance with the criteria described in the declaration of Helsinki and all patients provided written informed consent for their participation.

The primary study endpoint was a composite of cardiac death, non-fatal myocardial infarction, or unstable angina pectoris, requiring urgent coronary revascularization within 1-year follow-up. Study endpoints were adjudicated by a clinical event committee, blinded for any biomarker data, after the study was completed in 2015. In 2014, Roche Diagnostics GmbH offered the opportunity to determine GDF-15 with their pre-commercial assay in a limited number of BIOMArCS patients. Since no commercial GDF-15 assay would have been available within the foreseeable future, we decided to accept this one-time offer. We analyzed the blood samples of all patients with an investigator-reported endpoint event at that time, as well as 2 matched endpoint-free controls for each such event. Matching was based on admission hospital, age (± 5 year range), gender, diabetes mellitus, peripheral artery disease and history of coronary artery disease (CAD). We kept the results unanalyzed until study completion and event-adjudication. After study completion, it appeared that 37 of the investigator-reported events were confirmed as study endpoint. In the current analysis, these events were included as cases, together with their corresponding 74 matched controls.

Blood samples were initially handled and securely stored on-site. Aliquots were frozen at -80 degrees Celsius within two hours after withdrawal. Long-term storage and batchwise GDF-15 analysis took place at the department of Clinical Chemistry of the Erasmus MC, Rotterdam (the Netherlands). Laboratory personnel were blinded for any clinical data, including endpoint data. The plasma GDF-15 concentrations were measured using the quantitative sandwich electrochemiluminescence immunoassay "ECLIA" (Roche Diagnostics, Mannheim, Germany) on a Cobas e601 immunoassay analyzer. The lowest detection limit of GDF-15 analyte concentration was 400 pg/mL. No interference was found using in vitro tests to determine interference between 51 commonly used cardiovascular pharmaceuticals and the assay.

It is important to discern a fixed amount of individual biomarker variability from clinically relevant changes over time. Therefore certain parameters have been described to define individual variability, which are needed to interpret the relevance of changes of repeated measurements. The coefficient of variation (CV) of a series of measurements is defined as 100% times the standard deviation (sd) of the measurements divided by their mean value ($\overline{\mathrm{X}}$):

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

According to the methods by Fraser and Harris,[8] the total variation of a series of repeated measurements in individual subjects can be split in 3 components, which represent the variation due to the imprecision of the analytical process ($\mathrm{CV_a}$), the intra-individual or within-subject variation ($\mathrm{CV_i}$), and the inter-individual or between-subject variation ($\mathrm{CV_g}$). $\mathrm{CV_a}$ of GDF-15 in our laboratory appeared to be 1.75% and 1.88% for high and low concentrations, respectively. Subsequently, $\mathrm{CV_i}$ was defined as the median value of the

CVs of the repeated measurements in individual subjects ($CV_{subject}$), adjusted for the analytical variation:

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

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

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

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

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

Since a high II (>1.4) indicates a relatively high within-subject variation and low between-subject variation, it is more likely that an unusual biomarker value will lie outside the borders of most overlapping values and therefore population-based reference intervals are sufficient. Conversely, when the II is low (<0.6), it is agreed that subjects should have their own reference values, based on previous samples. The Reference Change Value (RCV) reflects the limit of (relative) change in biomarker values in individual subjects that can be explained by the combined within-subject and analytical variation. For biomarkers with a skewed distribution a log-normal approach has been described, and the RCV limits can be determined as follows:

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

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

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

Since GDF-15 is known to be initially elevated post ACS, all aforementioned variability parameters are based on > 30 day blood samples. Although the exact pathophysiological substrate for an initial elevation of GDF-15 in the acute phase of ACS is unknown,[9] a possible 'washout' effect due to an acute phase reaction is thereby hampered. After that period, biochemical as well as clinical stabilization are expected to be reached for adequately determining GDF-15 variability. Thereafter, only patients with at least three available measurements in that time window are included, leaving 20 cases and 46 controls for variability analysis.

Categorical data are presented as numbers and percentages. Continuous variables are presented as mean±standard deviation (SD) or as median and interquartile range (IQR), depending on their distribution. Normality of continuous variables was examined by visual inspection of the histogram and by normal Q-Q plots. The examined biomarker

GDF-15 (outcome) showed a skewed distribution and was therefore ^{2}log-transformed for further analyses. GDF-15 biomarker trajectories were examined across different follow-up time intervals after the ACS index event during one year of follow-up. Within the first 7 days from admission, each patient's maximum biomarker value was determined. The median values of the patient-level maximum were compared between the cases and controls by linear mixed effect (LME) models. Then, the patient-average biomarker trajectories between 7 and 30 days from admission and from 30 days onwards were compared between cases and controls by LME models with nested random effects. Time from index ACS event until each blood sample measurement and a group variable (case / control) were entered as fixed effects in the model, paired individuals as random effects and serial GDF-15 measurements as the dependent variable (model 1). Subsequently, we fitted multivariable LME models with adjustment for age and gender (model 2), and with additional adjustment for admission diagnosis, diabetes mellitus, smoking, hypertension, hypercholesterolemia, BMI, history of revascularization, history of myocardial infarction and serum creatinine value (which was measured at each sampling moment) (model 3). Values were eventually backtransformed to present mean differences (95% confidence intervals (CI)) between cases and controls on the linear scale. All data were analyzed with SPSS (version 21) and R statistical software (version 3.5.1). All statistical tests were two-tailed and p-values < 0.050 were considered statistically significant.

RESULTS

Baseline clinical characteristics are presented in Table 1. The matching procedure appeared successful, as there were no relevant differences between cases and controls, except for admission diagnosis of STEMI (p-value <0.001). During the first 7 days after the index ACS, GDF-15 levels reached maximum values (median [IQR]) of 2436 [2286 – 4236] pg/mL in cases and 1804 [1207 – 3749] pg/mL in the controls (p-value 0.22). These levels slightly decreased within the first 30 days, and the mean value within the 7 to 30 day period was 1908 pg/mL and 1590 pg/mL in cases and controls, respectively. This mean difference of 318 (95% CI ranging from -215 to 1058) pg/mL was statistically non-significant (p-value 0.26). From 30 days after the index ACS onwards until 1-year follow-up, cases had systematically higher GDF-15 levels than controls (Table 2, Figure 1). This difference remained significant after correction for age, gender and multiple cardiovascular risk factors (p-value 0.013). These findings are confirmed in strata according to gender, diabetes mellitus, smoking, serum creatinine value and admission diagnosis (Supplemental Tables 4 - 8). No differences were observed in GDF-15 levels across the various subgroups (all p-values for heterogeneity were > 0.05).

Table 1. Baseline clinical characteristics

	Cases (n = 37)	Controls (n = 74)	p-value
Age, years	67.9 ± 11.7	66.3 ± 11.2	0.79
Male gender, n (%)	30 (81.1)	60 (81.1)	0.95
Admission diagnosis, n (%)			
STEMI	13 (35.1)	42 (56.8)	<0.001
NSTEMI	18 (48.6)	27 (36.5)	0.17
UAP	6 (16.2)	5 (6.8)	0.78
Cardiovascular risk factors, n (%)			
Smoking			
Current	13 (35.1)	25 (33.8)	0.91
Former	11 (29.7)	23 (31.1)	0.97
Never	13 (35.1)	26 (35.1)	0.95
Diabetes Mellitus	12 (32.4)	26 (35.1)	0.52
Hypertension	19 (51.4)	40 (54.1)	0.77
Hypercholesterolemia	17 (45.9)	30 (40.5)	0.81
Medical history, n (%)			
Previous myocardial infarction	12 (32.4)	23 (29.7)	0.93
Previous PCI	11 (29.7)	20 (27.0)	0.88
Previous CABG	9 (24.3)	10 (13.5)	0.64
Previous stroke	8 (21.6)	7 (9.5)	0.78
History of peripheral vascular disease	10 (27.0)	13 (17.7)	0.73

Values are mean ± standard deviation or n (%). p-values were obtained by the linear mixed model (continuous variable) or generalized linear mixed model (categorical variable), whichever was appropriate.
CABG: coronary artery bypass grafting, NSTEMI: non-ST-segment elevation myocardial infarction, PCI: percutaneous coronary intervention, STEMI: ST-segment elevation myocardial infarction, UAP: unstable angina pectoris

Table 2. Mean GDF-15 (pg/mL) values in cases and controls in the 30 days to 1 year period after ACS admission

	Cases	Controls	Mean difference (95% CI)	p-value
Model 1*	1780	1414	366 (26, 788)	0.034
Model 2†	1744	1415	329 (2, 732)	0.049
Model 3‡	1704	1371	333 (68, 647)	0.013

* Unadjusted for patient characteristics
† Adjusted for age and gender
‡ Adjusted for age, gender, admission diagnosis, diabetes mellitus, smoking, hypertension, hypercholesterolemia, BMI, history of revascularization, history of myocardial infarction and serum creatinine value (measured at each time-point)

An overview of the different variability parameters, calculated for a selected amount of cases and controls, is presented in Table 3. With a CV_a of 2%, both groups displayed limited within-subject variability (CV_i of 16.3 for the cases and 11.5 for the controls), whereas the between-subject variability showed larger variation (CV_g of 73.1 for the cases and 62.0 for the controls). This is also shown by a plot (Figure 2), which illustrates low within-subject variability (CV_i / (CV_i + CV_g) = 16-18%) and large between-subject

variability (CV_g / (CV_i + CV_g) 82-84%) with a minimum of 579 pg/mL and a maximum of 9748 pg/mL. As could be expected from low within-subject variability and high between-subject variability in both groups, the II was low (below the threshold value of 0.6), and thus individual reference values are preferred. Thereby we found that the limits of change between subsequent measurements (RCV) are allowed to range from -36% to 57% in cases and from -28% to 38% in controls.

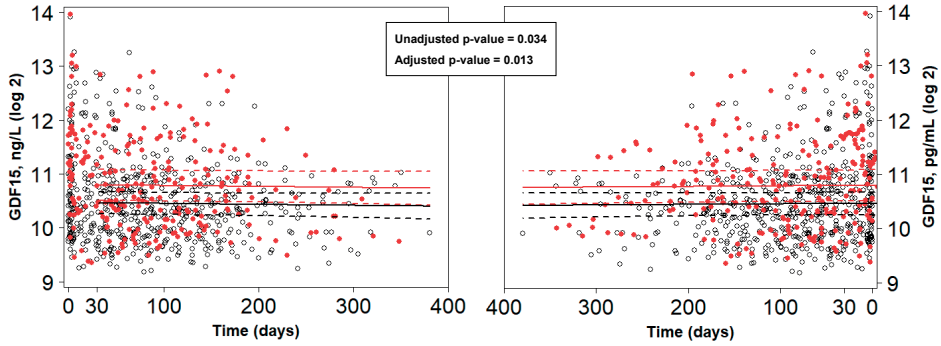

Figure 1. Serial measurements and temporal evolvement of GDF-15 (pg/mL) in cases (red) and controls (black).

The left graph shows the evolvement of GDF-15 since the index event (t=0) until 1-year follow-up. The right graph shows the evolvement of GDF-15 before the study endpoint (t=0 in cases), or until the last blood sample moment (t=0 in controls). The points represent measurements in individual patients. The lines represent the group average values (bold lines) and the 95% confidence intervals (dashed lines), using linear mixed models with nested random effects.

Table 3. Parameters describing the biological variability of GDF-15 serial measurements 30 days after the ACS index event in both cases and controls

	Cases (n = 20)	Controls (n = 46)
Average biomarker level (pg/mL), median [IQR]	1423 [1122 – 2594]	1317 [966 - 1705]
Analytical coefficient of variation (CV_a)	2%	2%
Intra-individual coefficient of variation (CV_i)	16.3	11.5
Inter-individual coefficient of variation (CV_g)	73.1	62.0
Index of individuality (II)	0.2	0.2
Reference change value (RCV)	45%	32%
Reference change value, upper limit	57%	38%
Reference change value, lower limit	-36%	-28%

Parameters describing the biological variability of GDF-15, as calculated by formulas presented in the method section.

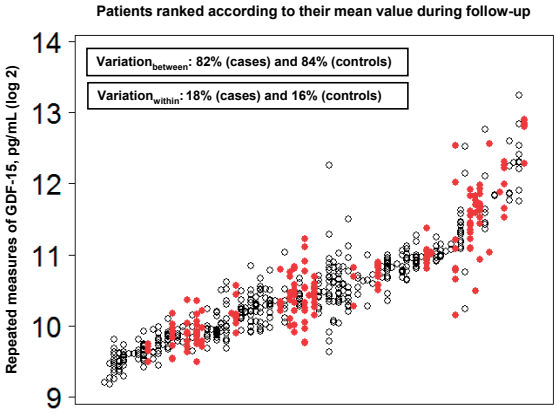

Figure 2. Graphical illustration of GDF-15 variability by displaying the distribution of GDF-15 measurements per patient 30 days after the ACS index event.

The data points represent measurements in individual patients (cases in red; controls in black), ranked according to their mean value during post 30 days follow-up in order to display within- and between-individual variation.

DISCUSSION

This is the first study to describe GDF-15 patterns in post ACS patients in great detail, utilizing a high-frequency blood sampling design during 1 year. Four key lessons were learned from our analysis. First, in individual patients, after reaching a peak value in the first week after admission, GDF-15 concentrations levelled off to levels that remain stable throughout 1-year follow-up. Second, importantly, there was no steady or sudden change in GDF-15 level prior to a recurrent event. Thus, no significant changes in GDF-15 values occurred after the initial post ACS phase. Third, patients who experienced a recurrent event had on average 26% higher GDF-15 levels than those who remained event-free. Although the prognostic value of GDF-15 has already been demonstrated by previous studies with one baseline measurement, we additionally proved that repeated post-discharge blood sampling of GDF-15 during 1 year might help improve accurate prognostication. Fourth, within-patient variability was much smaller than between-patient variability, meaning that the number of repeated blood samples to obtain a patient-specific stable GDF-15 level can be limited.

Considering the natural course of GDF-15 post ACS in our analysis, peak values are present in the first 7 days after an ACS, whereafter it seems that GDF-15 subtly reaches a stabilized phase without significant changes, especially prior to a recurrent event. By the use of frequent serial measurements, the stability of the marker in individual patients on the long term was established. This finding is supported by previous data in post ACS patients, demonstrating that GDF-15 concentrations show small alterations through the first 72 hours of hospitalization and potentially several months thereafter.[3,4]

The fact that GDF-15 concentration levels remain significantly higher in patients who experience a recurrent event than in event-free patients over the course of a year in our study without any level changes around the event, suggests that GDF-15 is not merely a reflection of extent of myocardial damage or infarct size, but rather reflects severity of (chronic) atherosclerotic disease burden at any time point. This proposition is further supported by findings with cardiovascular magnetic resonance, demonstrating that GDF-15 is unrelated to infarct size and myocardial area at risk 2-4 days after the index event.[9] Furthermore, GDF-15 concentrations on admission seemed to be similar between NSTEMI and STEMI patients, of whom more severe myocardial damage can be expected.[2,3] Thus, in support of our hypothesis, previous studies do not indicate that GDF-15 solely mirrors tissue damage.

With regard to prognostication, GDF-15 has been thoroughly investigated in clinical studies and shown to be an independent prognostic marker of mortality and cardiovascular events in both healthy individuals and CAD patients, which is in accordance with our results.[4-6,9-11] Specifically, a recent meta-analysis focused on ACS patients, including 8 studies and 8903 participants, showed a significant hazard ratio (95% confidence interval) of 1.66 (1.47 – 1.87) on the association between GDF-15 and mortality or recurrent MI.[6] However, most studies performed blood sampling only on admission at the onset of an ACS or at discharge during the recovery phase of an ACS. As we have demonstrated, initial GDF-15 peak values were largely present in the first 7 days after the index ACS, which is likely the expression of an acute phase reaction. Therefore, single blood samples timed in the early phase during the course of an ACS event may represent a peak level, which does not clarify its prognostic implications on long term post ACS. To our knowledge, only two clinical studies have performed a limited number of serial GDF-15 measurements in post NSTEMI patients.[3,4] Wollert et al[3] collected blood samples on admission and at 24, 48 and 72 hours in a subgroup of 399 patients, whereas Eggers et al[4] measured GDF-15 at baseline and after clinical stabilization at 6 weeks, 3 months, 6 months in 950 patients. Both studies found significant associations with respectively 1-year and 5-year mortality at each time point. Along with our data with highly frequent blood sampling, we have additionally demonstrated that obtained blood samples within a course of 1 year post ACS will provide comparable prognostic information.

The biological variability of GDF-15 in ACS patients has not been described so far. We found low within-subject variability and high between-subject variability, which corresponds with findings from a study on the biovariability of GDF-15 conducted in 41 patients with stable chronic systolic dysfunction.[12] In this study, GDF-15 was measured at four blood sampling time points up until 3 months and showed very little biological (within-)variation, while there was an elevated between-individual variation (reflected by a low II). Altogether, describing biomarker variability is warranted to provide insight into

the significance and interpretation of a biomarker in clinical practice. Our results indicate that changes in serial measurements of GDF-15 in an individual who experienced an ACS, independently of disease status (case or control), might be more useful than population derived reference values.

The unique design and character of this study enabled us to provide novel data on the temporal evolution and variability of GDF-15 post ACS. Nevertheless, some limitations warrant to be acknowledged. Due to the study design and its observational character, this substudy is unable to demonstrate causal inference. Whether GDF-15 merely reflects CAD pathways, or directly contributes to coronary pathophysiology remains unknown. Also, as opposed to previous studies with large cohorts, we could not demonstrate significant differences in GDF-15 levels between cases and controls within the first 30 days. This is probably due to a lack of power with a limited number of measurements < 30 days within a relatively small cohort. In line with this, we are aware of the fact that our study comprises a relatively small number of study patients and events. Further, by acknowledging previous studies that investigated the prognostic value of GDF-15 in large study populations, our study encompassing an exceptional blood sampling frequency method should rather be seen as hypothesis-testing with an extension to existing knowledge.

In conclusion, with detailed analysis of the longitudinal GDF-15 pattern post ACS, we have demonstrated that GDF-15 concentrations remain stable during follow-up with limited within-individual variation. In patients who eventually experience a recurrent event, GDF-15 is systematically elevated, independently of clinical risk factors and serum creatinine. Thus, to enable risk stratification with GDF-15 in post ACS patients, blood sampling might be used throughout the course of 1 year for prognostication, whereas the number of repeated sampling moments might be limited. Further exploration of the exact role of GDF-15 in risk stratifying post ACS patients and deciding on clear cut off points is warranted in future studies in order to make accurate prognostications.

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SUPPLEMENTARY APPENDIX

Table 4. GDF-15 (pg/mL) values of cases and controls in strata according to gender in the 30 days to 1 year period after index ACS

	Men			Women			P for heterogeneity
	Cases	Controls	Mean difference (95% CI)	Cases	Controls	Mean difference (95% CI)	
Model 1*	1822	1446	377 (-10, 867)	1512	1185	326 (-251, 1262)	0.97
Model 2†	1806	1452	354 (-15, 817)	1505	1227	279 (-281, 1170)	0.96
Model 3‡	1773	1329	444 (142, 809)	1506	1619	-113 (-596, 598)	0.10

Mean GDF-15 values were based on nested linear mixed effects models with (^{2}log-transformed) GDF-15 as the dependent variable, and with gender, time from index ACS event to the blood sample measurement and group variable (case / control) as the main independent variables. To obtain the p-value for heterogeneity, an interaction term (group variable * gender) was added to the model.

* Unadjusted for patient characteristics

† Adjusted for age

‡ Adjusted for age, admission diagnosis, diabetes mellitus, smoking, hypertension, hypercholesterolemia, body mass index, history of revascularization, history of myocardial infarct and serum creatinine value (measured at each time-point)

Table 5. GDF-15 (pg/mL) values of cases and controls in strata according to diabetes mellitus in the 30 days to 1 year period after index ACS

	Diabetes			Non-diabetes			P for heterogeneity
	Cases	Controls	Mean difference (95% CI)	Cases	Controls	Mean difference (95% CI)	
Model 1*	2131	1816	315 (-324, 1228)	1605	1208	397 (37, 860)	0.57
Model 2†	2038	1772	266 (-324, 1096)	1607	1236	371 (22, 815)	0.56
Model 3‡	2175	1760	415 (-169, 1213)	1508	1215	293 (9, 643)	0.98

Mean GDF-15 values were based on nested linear mixed effects models with (^{2}log-transformed) GDF-15 as the dependent variable, and with presence of diabetes mellitus, time from index ACS event to the blood sample measurement and group variable (case / control) as the independent variables. To obtain the p-value for heterogeneity, an interaction term (group variable * diabetes mellitus) was added to the model.

* Unadjusted for patient characteristics

† Adjusted for age and gender

‡ Adjusted for age, gender, admission diagnosis, smoking, hypertension, hypercholesterolemia, body mass index, history of revascularization, history of myocardial infarct and serum creatinine value (measured at each time-point)

Table 6. GDF-15 (pg/mL) values of cases and controls in strata according to smoking in the 30 days to 1 year period after index ACS

	Smoking			Non-smoking			P for heterogeneity
	Cases	Controls	Mean difference (95% CI)	Cases	Controls	Mean difference (95% CI)	
Model 1*	1410	1265	145 (-294, 784)	1997	1458	539 (65, 1158)	0.38
Model 2†	1518	1392	126 (-326, 771)	1889	1411	478 (50, 1411)	0.36
Model 3‡	1832	1552	280 (-226, 978)	1646	1317	330 (-31, 791)	0.78

Mean GDF-15 values were based on nested linear mixed effects models with (^{2}log-transformed) GDF-15 as the dependent variable, and with smoking status, time from index ACS event to the blood sample measurement and group variable (case / control) as the independent variables. To obtain the p-value for heterogeneity, an interaction term (group variable * smoking status) was added to the model.

* Unadjusted for patient characteristics

† Adjusted for age and gender

‡ Adjusted for age, gender, admission diagnosis, diabetes mellitus, hypertension, hypercholesterolemia, body mass index, history of revascularization, history of myocardial infarct and serum creatinine value (measured at each time-point)

Table 7. GDF-15 (pg/mL) values of cases and controls in strata according to creatinine levels (µmol/L) in the 30 days to 1 year period after index ACS

	Creatinine ≥ 85			Creatinine < 85			P for heterogeneity
	Cases	Controls	Mean difference (95% CI)	Cases	Controls	Mean difference (95% CI)	
Model 1*	1952	1532	420 (-79, 1091)	1490	1288	203 (-220, 794)	0.68
Model 2†	1640	1518	122 (-92, 977)	1545	1319	226 (-193, 800)	0.78
Model 3‡	1817	1460	357 (-25, 841)	1553	1336	217 (-151, 698)	0.71

Mean GDF-15 values were based on nested linear mixed effects models with (^{2}log-transformed) GDF-15 as the dependent variable, and with serum creatinine level (dichotomized into equal or above the median and below the median), time from index ACS event to the blood sample measurement and group variable (case / control) as the independent variables. To obtain the p-value for heterogeneity, an interaction term (group variable * (dichotomous) serum creatinine level) was added to the model.

* Unadjusted for patient characteristics

† Adjusted for age and gender

‡ Adjusted for age, gender, admission diagnosis, smoking, diabetes mellitus, hypertension, hypercholesterolemia, body mass index, history of revascularization and history of myocardial infarction

Table 8. GDF-15 (pg/mL) values of cases and controls in strata according to admission diagnosis in the 30 days to 1 year period after index ACS

	STEMI			NSTEMI			P for heterogeneity
	Cases	Controls	Mean difference (95% CI)	Cases	Controls	Mean difference (95% CI)	
Model 1*	1535	1223	312 (-119, 911)	1758	1792	-34 (-502, 604)	0.29
Model 2†	1573	1275	299 (-123, 876)	1746	1725	20 (-428, 624)	0.36
Model 3‡	1670	1337	333 (-68, 862)	1748	1485	264 (-140, 789)	0.76

Mean GDF-15 values were based on nested linear mixed effects models with (^{2}log-transformed) GDF-15 as the dependent variable, and with admission diagnosis, time from index ACS event to the blood sample measurement and group variable (case / control) as the independent variables. To obtain the p-value for heterogeneity, an interaction term (group variable * admission diagnosis) was added to the model.

* Unadjusted for patient characteristics

† Adjusted for age and gender

‡ Adjusted for age, gender, smoking, diabetes mellitus, hypertension, hypercholesterolemia, body mass index, history of revascularization, history of myocardial infarct and serum creatinine value (measured at each time-point)

Chapter 8

Persistently elevated levels of sST2 after acute coronary syndrome are associated with recurrent cardiac events

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Submitted

ABSTRACT

Background

Elevated soluble Suppression of Tumorigenicity-2 (sST2) levels at admission is associated with adverse clinical outcome in patients admitted for acute coronary syndrome (ACS). We studied the dynamics of sST2 over time in post-ACS patients prior to a recurrent ACS.

Methods

For our analysis, we used the BIOMArCS case-cohort consisting of 187 patients who underwent serial blood sampling during one year follow-up post-ACS. sST2 was batch-wise quantified after completion of follow-up in a median of 8 (IQR: 5-11) measurements per patient. Linear mixed effect models were used to describe the average washout patterns. Joint-modelling was used to investigate the association between the longitudinally measured biomarkers and recurrent ACS, expressed in hazard ratios (aHR) adjusted for gender, GRACE risk score and history of cardiovascular diseases.

Results

Median age was 64 years and 79% were men. Patients with the endpoint had systematically higher sST2 level than those that remained endpoint-free (29.6 ng/ml versus 33.3 ng/ml, p-value 0.052). The aHR for the endpoint per standard deviation increase of sST2 was 1.64 (95% confidence interval: 1.09-2.34; p=0.019) at any time point. We could not identify a steady or sudden increase of sST2 in the run-up to the study endpoint.

Conclusion

Asymptomatic post-ACS patients with persistently elevated sST2 levels are at higher risk of fatal or non-fatal reACS during 1 year follow-up.

INTRODUCTION

Suppression of Tumorigenicity-2 (ST2) is an interleukin (IL) 1 receptor that binds to its ligand IL-33. In response to cardiac stress, soluble ST2 (sST2) and IL-33 are up-regulated. However, whereas IL-33 induces protective inflammatory pathways reducing adverse remodeling of cardiomyocytes through ST2, sST2 serves as a decoy receptor to IL-33, preventing these processes.[1] Indeed, studies have demonstrated that elevated sST2 level at admission is associated with adverse clinical outcome in heart failure patients, as well as in those admitted for acute coronary syndrome (ACS).[2, 3] Since ACS patients are at increased risk of recurrent cardiac events, especially during the first year, it is worthwhile to study the dynamics of sST2 over time. Particularly, repeated post-discharge sST2 measurements may detect episodes of increased coronary vulnerability in ACS patients, and may thus help to improve prognostication. We evaluated this hypothesis in a series of patients who were discharged after ACS and, and who underwent high-frequency blood sampling during one year.

METHODS

Study design

We studied post-ACS patients who participated in BIOMArCS (BIOMarker study to identify the Acute risk of a Coronary Syndrome).[4, 5] BIOMArCS is a multicenter (18 hospitals in The Netherlands) prospective study that included patients admitted with an ACS, aged 40 years or older and with at least 1 prespecified CV risk factor, to study biomarker evolutions using serial blood sampling during one year of follow-up. The in total 844 enrolled patients underwent venepuncture at regular intervals: every two weeks during the first six months of follow-up and monthly thereafter. A patient's venepuncture schedule was discontinued after coronary artery bypass grafting, an hospital admission for heart failure or in case of a (sudden) decline in renal function to a glomerular filtration rate (eGFR) <30mL/min/1.73m^2, to avoid biased biomarker levels. The study endpoint comprised of cardiac death, myocardial infarction, or unstable angina requiring urgent coronary revascularization, and was eventually reached by 45 patients.

We performed a case-cohort analysis within BIOMArCS. The details of the selection methods are described elsewhere.[DiB] Briefly, we randomly selected 150 patients from the full cohort, and added the 37 study endpoint cases that were outside this random selection. Hence, the analysis dataset consisted of all 45 endpoint cases and 142 event-free patients.

The study was approved by the medical ethics committees of the participating hospitals, conducted in accordance with the Declaration of Helsinki, and registered in the

Netherlands Trial register (NTR1698). All patients signed informed consent for their participation in the study.

sST2 measurements

On-site obtained blood samples were stored at -80 degrees Celsius after sample preparation within 82 (25th-75th percentile 58-117) minutes post withdrawal. Subsequently, all samples were securely transported to the Erasmus MC for long-term storage. Serum samples were used to batch-wise quantify sST2 levels (Presage ST2 assay, Critical diagnostics, San Diego, California, USA), blinded for patient characteristics and outcome. The lower limit of detection was 1.31ng/ml with reference values of 8.5-49.3 ng/ml for man and 7.1-33.5ng/ml for women; the analytical coefficient of variation was <5%.

For the current analysis, median 8 (25th-75th percentile 5-11) serial samples were available per patient (altogether 1282 samples).

Statistical analysis

We used the framework of joint models for longitudinal and survival data to investigate the incremental value of repeated sST2 measurements for predicting reACS.[7] In these joint models, a linear mixed-effects (longitudinal) model was used to estimate sST2 trajectories for each patient, adjusted for GRACE risk (including age), sex and history of cardiovascular diseases. The longitudinal models were then combined with Cox proportional hazards models to study the association of sST2 with the study endpoint. The result of the joint-model is presented as an adjusted hazard ratio (aHR) with 95% confidence interval per standard deviation increase in sST2 level (on the log-scale).

Analyses were performed with R Statistical Software, in particular the packages *nlme* and *JMbayes*. Statistical tests were two-tailed and a p-value of 0.05 was used as threshold of statistical significance.

RESULTS

The median age was 64 years (25th-75th percentile 55.3-71.6), and 79% were men. Patients had a CCS Angina Grading Scale ≤1 at 95% of the sample moments and a NYHA classification ≤1 at 93% of the sample moments, reflecting clinical stability. The average eGFR per patient, calculated using all sample moments, was 90.9 (25th-75th percentile 72.4-111.4) mL/min/1.73m^2. Clinical characteristics of the patients are described in Table 1.

sST2 was elevated at the index ACS and subsequently stabilized within 7 days. In the post 7-day period, cases had systematically higher sST2 level than non-cases (29.6 ng/ml versus 33.3 ng/ml, p-value 0.052). The aHR for the endpoint per standard devia-

Table 1. Patient characteristics of the case-cohort

	Endpoint cases	Endpoint-free patients	p-value
Number of patients	45	142	
Age, yr (IQR)	67.4 (57.1-76.5)	62.6 (55.0-70.9)	0.075
Man (%)	36 (80.0)	111 (78.2)	0.79
Cardiovascular risk factors (%)			
Diabetes Mellitus	17 (37.8)	24 (16.9)	0.003
Hypertension	22 (48.9)	77 (54.2)	0.53
Hypercholesterolemia	20 (44.4)	72 (50.7)	0.46
Current smoker	17 (37.8)	60 (42.2)	0.52
History of cardiovascular disease (%)			
Myocardial infarction	14 (31.1)	43 (30.3)	0.92
Coronary artery bypass grafting	11 (24.4)	12 (8.5)	0.004
PCI	14 (31.1)	38 (27.0)	0.59
Stroke	9 (20.0)	16 (11.3)	0.13
Peripheral vessel disease	10 (22.2)	9 (6.3)	0.004
Admission diagnosis (%)			0.46
STEMI	16 (35.6)	65 (45.8)	
NSTEMI	22 (48.9)	56 (39.4)	
Unstable angina pectoris	7 (15.6)	21 (14.8)	
PCI performed	34 (87.2)	109 (82.6)	0.50
Physical examination			
GRACE risk score (IQR)	121 (98-141)	109 (88-130)	0.022
Body mass index (SD)	27.2 (3.7)	27.8 (3.8)	0.36
Heart rate (SD)	75 (16)	73 (17)	0.59
SBP (SD)	145 (24)	138 (27)	0.095
DBP (SD)	72 (3)	81 (17)	0.48
Discharge medication (%)			
Aspirin	45 (100)	132 (93.0)	0.20
P2Y12 inhibitor	44 (96.8)	128 (90.4)	0.37
Vitamin K antagonist	5 (9.7)	11 (7.9)	0.57
Statin	44 (96.8)	136 (95.6)	0.46
Beta-blocker	42 (93.5)	121 (85.1)	0.72
Ace inhibitor or ARB	41 (90.3)	120 (84.2)	1.00

Normally distributed continuous variables are presented as mean with SD, non-normally distributed continuous variables are presented as median with IQR. Categorical variables are presented as numbers and percentages.
ACE: angiotensin converting enzyme, ARB: angiotensin II receptor blocker, CAD: coronary artery disease, DBP: diastolic blood pressure, GRACE risk score: Global Registry of Acute Coronary Events risk score, NSTEMI: non-STEMI, PCI: Percutaneous coronary intervention, SBP: systolic blood pressure, STEMI: ST-elevation myocardial infarction, yr: year

tion increase was 1.64 (95% confidence interval: 1.09-2.34; p=0.019) at any time point. We could not identify a steady or sudden increase of sST2 in the run-up to the study endpoint (Figure). Repeatedly measured serum levels resulted in slightly improved

discrimination between cases and non-cases compared to a randomly selected *single* measurement taken during the follow-up, but this difference did not reach statistical significance (C-index 0.59 vs 0.57, p=0.53).

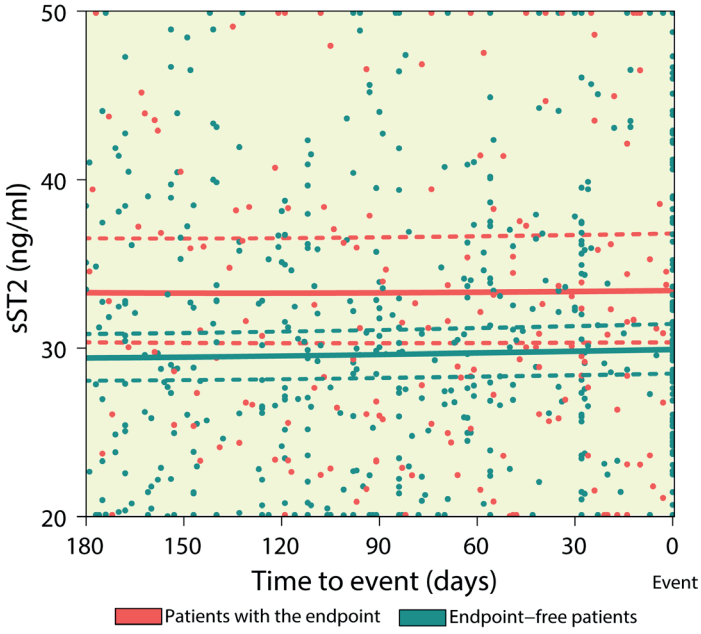

Figure. Average sST2 levels
The X-axis depicts time *until* event. Hence, day zero is the time of event or censoring. The thick line shows the average value of sST2 in either patients in whom the endpoint occurred (red), or endpoint-free patients (green).

DISCUSSION

Post-ACS patients with persistently elevated sST2 levels are at higher risk of fatal or non-fatal reACS during 1 year follow-up. Moreover, as intrapatient variability is low, a limited number of measurements may suffice for prognostication.

sST2 is upregulated in myocytes during cardiac stress and advances adverse cardiac remodeling. [1] Previously, it has been shown that the serum level of sST2 is associated with the extend of myocardial injury caused by an ACS one day after an ACS event.[8] Our findings now suggest that persistent elevated sST2 levels promote adverse remodeling and the development of reACS during the first year after an ACS event. Both mechanical strain as well as inflammatory cytokines (i.e. interleukin-1β) are able to upregulate sST2 in myocytes.[8-10] It may be hypothesized that the myocardium of patients with persistently high levels of sST2 are exposed to more cardiac stress and inflammation over time than patients with low sST2 levels. Potentially this constant state

of stress and inflammation may affect the risk of novel adverse cardiac events such as ACS or cardiac death.

The prognostic role of sST2 in ACS has received more attention in recent years.[2, 3, 11-14]. A study by Kohli et al among 4426 patients with non-ST-elevation ACS revealed that patients with a high baseline sST2 level were more likely to have heart failure or die of cardiovascular causes at 30 days and 1 year.[2] This association was independent from clinical covariates and other baseline biomarkers such as NT-proBNP. In addition, in ST-elevation myocardial infarction, baseline sST2 was also found to be an independent predictor of cardiac death within 30 days after an MI event. It was concluded that within 30 days post an MI, sST2 level carries additional (novel) prognostic information over just left ventricle wall stress (as indicated by NT-proBNP).[3] Lastly, O'Malley et al showed in 4432 non-ST-elevation ACS patients that baseline sST2 level significantly predicts 1-year heart failure, but does not independently predict myocardial infarction or recurrent ischemia.[13]

Baseline measurements of markers of hemodynamic stress, such as sST2, may not reflect atherosclerotic disease and are therefore better predictors of all-cause mortality and heart failure than thromboembolic events such as an ACS. However, as it has been shown that baseline sST2 level is able to independently predict cardiac death, sST2 may be of value to the currently established cardiac markers. Moreover, as our study now shows that persistently high sST2 levels during one year post ACS may be used to identify high-risk patients, future studies may assess if repeated sST2 level may be suitable for a multi-marker approach to stratify ACS-patients according to their risk of 1 year adverse outcome.

So far the dynamics of sST2 after ACS and prior to a recurrent ACS had not yet been investigated. Our study is distinctive in that we obtained a large number (median 8 per patient) of repeated measurements to provide a detailed description of the temporal evolution of sST2 in individuals during the first year after ACS admission. As such, our findings are accurate and robust. However, our study was limited with respect to the number of patients who reached the study endpoint. Hence, we were unable to adjust the observed relation between sST2 and recurrent ACS for other prognostic markers and for residual confounders.

In conclusion, asymptomatic post-ACS patients with persistently elevated sST2 levels are at increased risk of recurrent ACS. The role of sST2 for risk prediction in patients with clinically stable CAD warrants further investigation.

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Chapter 9

MAPK-cascade stimulating biomarkers in relation to recurrent coronary events following an acute coronary syndrome

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ABSTRACT

Background

The intracellular mitogen-activated protein kinase (MAPK) cascade regulates intracellular processes that modulate cardiovascular disease progression. We explored the time-course of serum biomarkers that stimulate the MAPK-cascade in post-acute coronary syndrome (ACS) patients, prior to a recurrent coronary event.

Methods

BIOMArCS is a high-frequent repeated blood sampling study in post-ACS patients. We performed a nested case-control study selecting the 45 patients who experienced a recurrent event (cases) and 2 matched event-free controls per case during 1-year follow-up. Olink Proteomics 'immunoassay was used to measure 25 serum biomarkers. Results are expressed in the arbitrary Normalized Protein eXpression (NPX) unit on the 2log-scale. Linear mixed-effects models were applied to examine time-courses and differences between cases and controls.

Results

Mean age was 66±12 years and 80% were men, with no differences between cases and controls. Early cases had significantly higher levels of ANG-1 (difference 0.95 NPX (95%CI 0.36-1.54), PAR-1 (difference 0.50 NPX (95%CI 0.22-0.77) and BMP-6 (difference 0.55 NPX (95%CI 0.21-0.90) than controls. No differences in biomarker levels were observed between late cases and matching controls. In particular, in cases, no increase was observed prior to the moment of the recurrent event.

Conclusion

Patients with an early recurrent coronary event after an index-ACS had higher levels of ANG-1, PAR-1 and BMP-6 than patients who remained event-free.

INTRODUCTION

Although outcome for patients with cardiovascular disease (CVD) has improved over the last decades, hospitalization rates are still increasing.[1-3] This increase can partly be explained by the increasing population of patients who survived an acute coronary syndrome (ACS) and who are at risk of experiencing a recurrent coronary event.[1] As CVD is dynamic and shows considerable inter-patient variation, to improve secondary prevention, insight in the course of CVD in individual subjects is required. Since biomarker profiles may serve as a proxy for CVD status and development, the exploration of (established and) evolving markers covering relevant pathophysiological processes is warranted.

The mitogen-activated protein kinase (MAPK) cascade, is an intracellular cascade of proteins that enables extracellular stimuli – blood biomarkers - to modulate several intracellular processes i.e. cell growth, differentiation, proliferation, and apoptosis.[4] Basic research has shown that the MAPK-cascade plays a pivotal role in cellular processes that advance CVD progression.[5] First, the MAPK-cascade promotes atherosclerotic lesion formation, i.e. by inducing inflammation and cell apoptosis. Secondly, it may activate pathological cardiac remodeling after myocardial infarction by constraining myocyte mitosis and promoting fibrosis. Thirdly, the cascade might be directly involved in the development of in-stent restenosis.[5]

In view of the pivotal role of the MAPK-cascade in the development and progression of CVD, blood biomarkers that regulate the cascade may be useful for the identification of patients with CVD who are at higher risk of developing a (recurrent) coronary event. However, translational research relating the MAPK-cascade to clinical CVD progression is scarce.[5]

We aimed to explore the course of protein blood biomarkers that stimulate the intracellular MAPK-cascade in post-ACS patients prior to the development of a recurrent coronary event during one year of follow-up.

METHODS

Study population

We performed a case-control study that is embedded in The 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS).[6] BIOMArCS is a multicenter observational study with a unique high-frequency sampling design, to study the course of blood biomarkers in patients following an ACS in anticipation of a recurrent event. The design of BIOMArCS has been described in detail elsewhere.[6] In brief, BIOMArCS enrolled 844 patients with ACS, aged ≥40 years and who had at least 1 pre-specified

cardiovascular risk factor. After enrolment, venipuncture was performed at admission, discharge, and subsequently every two weeks during the first half-year and every month during the second half-year. A median number of 17 repeated blood samples per patient were obtained.

BIOMArCS was approved by the Institutional Review Boards of all enrolling hospitals, and all participating patients provided written informed consent. BIOMArCS is registered in The Netherlands Trial Register NTR1698 and NTR1106.

Case-control design

The current analysis is based on a case-control approach. A total of 45 patients (cases) in BIOMArCS reached the composite study endpoint of cardiac death, non-fatal myocardial infarction (MI), or unstable angina (UA) requiring urgent coronary revascularization during one year of follow-up after the index-ACS. These cases were with two controls that are selected from BIOMArCS event-free patients. Cases and controls were matched on age, sex and admitted hospital. For reasons of efficiency, for each case, the blood sample at hospital admission and the last and second last samples prior to the recurrent coronary event have been analyzed. In controls, we selected the blood sample at hospital admission and the blood sample that corresponds in time with the recurrent event of the matched case.

As a pragmatic choice, separate analyses were performed for cases (and their matching controls) that experienced their event in the first 30 days after the index-ACS, and for cases (and their matching controls) that experienced their event thereafter. Hence, we were able to differentiate between the behavior of biomarkers during the acute and post-stabilization phase after the index-ACS.

Biomarker measurements

Targeted protein biomarker measurements were performed by the Proximity Extension Assay (PEA) Technique using Olink Proteomics' CVD II panel (Olink Proteomics AB, Uppsala, Sweden). Details concerning PEA and the CVD II panel are described on the website of Olink Proteomics (www.olink.com). In brief, the PEA technique consists of a pair of oligonucleotide-labelled antibody probes that pair-wise bind to a targeted protein biomarker in a blood sample. This binding induces amplification of the protein biomarker by real-time PCR (Fluidigm® BioMark™ HD System).

The PEA technique enables simultaneous analysis of all protein biomarkers of the CVD II panel in one blood sample. Olink proteomics' CVD II panel provides measurements of 25 protein blood biomarkers that are related to the intracellular MAPK-cascade (overview of proteins in Table 1). Every measured protein blood biomarker is expressed in an arbitrary unit on the log2-scale called Normalized Protein eXpression (NPX). Accordingly, an increase or decrease of *one* NPX corresponds with a doubling or halving

of a biomarker serum level. NPX values cannot be compared across different proteins. For each protein biomarker, general calibrator curves to calculate approximate concentrations are available on the website of Olink Proteomics.

Table 1. Overview of the assessed protein biomarkers

Abbreviation	Full name	Synonyms	Molecular function
NEMO	NF-kappa-B essential modulator	IKBKG, FIP3	Binding protein
HB-EGF	Proheparin-binding EGF-like growth factor	DTR, DTS, HEGFL	Growth factor/receptor
SCF	Stem cell factor	KITLG,MGF	Cytokine/growth factor
PDGF subunit B	Platelet-derived growth factor subunit B	PDGFB,PDGF2, SIS	Developmental protein/growth factor
GDF-2	Growth/differentiation factor 2	BMP9	Cytokine/growth factor
ANG-1	Angiopoietin-1	ANGPT1	Developmental protein
CCL3	C-C motif chemokine 3	MIP1A, SCYA3, G0S19-1	Chemokine
TIE2	Angiopoietin-1 receptor	TEK, VMCM, VMCM1	Receptor
PAR-1	Proteinase-activated receptor 1	F2R, CF2R, TR	Receptor
LEP	Leptin	OB, OBS	Hormone/growth factor
REN	Renin		Hydrolase
TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A	RANK, ODFR, CD265, NFKB activator	Receptor
THPO	Thrombopoietin / megakaryocyte colony-stimulating factor	MGDF	Cytokine/hormone
FGF-21	Fibroblast growth factor 21		Growth factor
GAL-9	Galectin-9	LGALS9	Binding protein
SRC	Proto-oncogene tyrosine-protein kinase SRC	SRC1, C-SRC	Kinase
GH	Growth hormone	GH1, somatotropin	Hormone
XCL1	X-C motif chemokine ligand 1	Lymphotactin, LTN, SCYC1, ATAC, SCM-1	Chemokine
FGF-23	Fibroblast growth factor 23	HYPF	Growth factor
CCL17	C-C motif chemokine 17	SCYA17, TARC	Chemokine
IL-18	Interleukin-18	IGIF, IL1F4	Cytokine
BMP-6	Bone morphogenetic protein 6	VGR1, VGR	Cytokine/developmental protein/growth factor
IL-6	Interleukin-6	IFNB2, BSF2, CDF, HGF	Cytokine/growth factor
AMBP	alpha-1-microglobulin/bikunin precursor	HCP, ITIL, ITI, Bikunin, EDC1, Trypstatin	Protease inhibitor
CD40-L	CD40 ligand	CD40LG, TNFSF5, TRAP, HIGM1, CD154	Cytokine

Statistical analysis

Continuous variables are presented as medians with interquartile range (IQR) and categorical variables as numbers with percentages. Differences between cases and controls were compared with Mann-Whitney U and Pearson Chi-square tests, respectively.

As indicated, all biomarkers were analyzed on a log2-transformed scale. We fitted a linear mixed-effects model for every biomarker to describe patient-specific longitudinal

biomarker trajectories as a function of time. Likelihood ratio tests and F-tests were used for hypothesis testing, whereas residuals were used to examine the model assumptions.

We considered 25 biomarkers. To adjust for inflation of the type I error with multiple testing, statistical significance was stated at p=0.003 (two-tailed test), based on the matrix spectral decomposition method.[7] R statistical software (version 3.4.0) was used for analyses, in particular the package nlme (https://cran.r-project.org/web/packages/nlme/index.html).

RESULTS

Baseline characteristics

Mean age of all patients was 66.0 ±11.9 years and 80.2% were men. Cases and controls did not show any significant differences in presentation, initial treatment, cardiovascular risk factors and medication at first blood sample, indicating successful matching (Table 2).

Table 2. Baseline clinical characteristics

	Cases n=44	Controls n=87	p-value
Presentation and initial treatment			
Men	35 (79.5)	70 (80.5)	0.90
Age - yr	67.5 (57.3-77.5)	66.7 (57.4-75.5)	0.83
Admission diagnosis			0.38
STEMI	16 (36.4)	42 (48.3)	
NSTEMI	22 (50.0)	33 (37.9)	
UAP	6 (13.6)	12 (13.8)	
CAG performed	39 (88.6)	82 (94.3)	0.25
PCI performed	33 (86.8)	67 (81.7)	0.48
CKmax - U/L	418 (195-1142)	513 (169-1332)	0.94
Cardiovascular risk factors			
Smoking			0.81
Current	17 (38.6)	35 (40.2)	
Former	12 (27.3)	27 (31.0)	
Never	15 (34.1)	25 (28.7)	
Diabetes mellitus	16 (36.4)	32 (36.8)	0.96
Hypertension	21 (47.7)	44 (50.6)	0.76
Hypercholesterolemia	19 (43.2)	46 (52.9)	0.30
Creatinine - µmol/L	88 (73-93)	81 (67-97)	0.15
Cardiovascular history			
Peripheral arterial disease	10 (22.7)	7 (8.0)	0.018
Myocardial infarction	14 (31.8)	33 (37.9)	0.49

Table 2. Baseline clinical characteristics (*continued*)

	Cases	Controls	
	n=44	n=87	p-value
PCI	14 (31.8)	29 (33.3)	0.86
CABG	10 (22.7)	17 (19.5)	0.67
Stroke	9 (20.5)	5 (5.7)	0.010
Valvular heart disease	4 (9.1)	3 (3.4)	0.18
Heart failure	4(9.1)	1 (1.1)	0.025
*Medication at first blood sample moment >7 days after the index ACS**			
Aspirin	35 (92.1)	76 (92.7)	0.91
P2Y12 inhibitor	36 (94.7)	74 (90.2)	0.41
Vitamin K antagonist	7 (18.4)	8 (9.8)	0.18
Statin	35 (92.1)	79 (96.3)	0.32
Beta-blocker	36 (94.7)	69 (84.1)	0.10
ACE inhibitor or ARB	34 (89.5)	65 (79.3)	0.17

Continuous variables are presented as median (25th-75th percentile). Categorical variables are presented as number (percentage).

* The first blood sample >7 days was taken at a median (25th-75th percentile) of 24 (16-34) days after the index ACS.

ACE: angiotensin converting enzyme, ARB: angiotensin II receptor blocker, CABG: coronary artery bypass grafting, CKmax: maximum creatine kinase during the index admission, NSTEMI: non-ST-elevation myocardial infarction, PCI: percutaneous coronary intervention, STEMI: ST-elevation myocardial infarction, Troponin ax: maximum troponin value during the index admission, UAP: unstable angina pectoris, yr: years

Biomarker trajectories in the first 30 days after the index ACS

15 Cases reached the study endpoint within 30 days after the index ACS. They had higher serum levels of ANG-1 (difference of 0.95 NPX, 95% confidence interval [CI] 0.36-1.54), PAR-1 (difference of 0.50 NPX, 95%CI 0.22-0.77) and BMP-6 (difference of 0.55 NPX, 95%CI 0.21-0.90) than the matched controls (Table 3, Figure 1 left-hand panel).

Biomarker trajectories after 30 days

30 Cases had the study endpoint in >30 days after the index ACS. Interestingly, in these late cases, the biomarker levels during the first 30 days after the index ACS tended to be lower than in the early cases (Table 4). In the post-30 day time window, cases and matched controls appeared to have similar levels of biomarkers (Table 5). Importantly, we found no steady or sudden increase in biomarkers in the days or weeks prior to the recurrent event.

Table 3. Difference in biomarker serum level between cases and controls ≤30 days

Biomarker (NPX)	Coefficient	95%CI	p-value
NEMO	1.16	(0.36-1.95)	0.005
HB-EGF	0.61	(0.11-1.12)	0.018
SCF	-0.13	(-0.59-0.32)	0.55
PDGF subunit B	0.92	(0.31-1.54)	0.004
GDF-2	-0.063	(-0.35-0.23)	0.66
ANG-1	0.95	(0.36-1.54)	0.002
CCL3	0.29	(-0.12-0.71)	0.16
TIE2	0.12	(-0.048-0.29)	0.16
PAR-1	0.50	(0.22-0.77)	<0.001
LEP	0.35	(-0.31-1.00)	0.29
REN	0.45	(-0.18-1.08)	0.16
TNFRSF11A	0.41	(0.003-0.82)	0.048
THPO	0.22	(-0.018-0.45)	0.069
FGF-21	0.55	(-0.39-1.49)	0.24
GAL-9	0.16	(-0.083-0.41)	0.19
SRC	0.29	(-0.18-0.76)	0.22
GH	0.71	(-0.61-2.02)	0.29
XCL1	0.34	(0.018-0.66)	0.039
FGF-23	0.90	(0.16-1.63)	0.018
CCL17	0.77	(0.089-1.46)	0.028
IL-18	0.19	(-0.15-0.52)	0.27
BMP-6	0.55	(0.21-0.90)	0.002
IL-6	0.95	(0.074-1.82)	0.034
AMBP	0.11	(-0.033-0.25)	0.13
CD40-L	1.32	(0.41-2.22)	0.006

For every biomarker, the difference in biomarker serum level between cases and controls is expressed in a relative arbitrary unit on the log 2 scale. Thus, an increase or decrease of one NPX corresponds with a doubling or a halving of the protein biomarker serum level.
ACS: acute coronary syndrome, CI: confidence interval, NPX: Normalized Protein eXpression

DISCUSSION

Serum levels of ANG-1, PAR-1 and BMP-6 were significantly higher in patients who developed a recurrent coronary event within the first 30 days following an ACS than in their matching controls. In the time period >30 days after the index-ACS until 1 year follow-up, patients with and without a recurrent coronary event had similar patterns of MAPK stimulating biomarkers.

Our study results suggest that during the first 30 days post-ACS, the initial ACS induces numerous stimuli that activate the intracellular MAPK-cascade, which, in turn, may induce a pro-inflammatory and thrombogenic state, leading to a recurrent event. Potentially, other processes play a more important role in the initiation of a new coronary event following stabilization after the first 30 days post-ACS

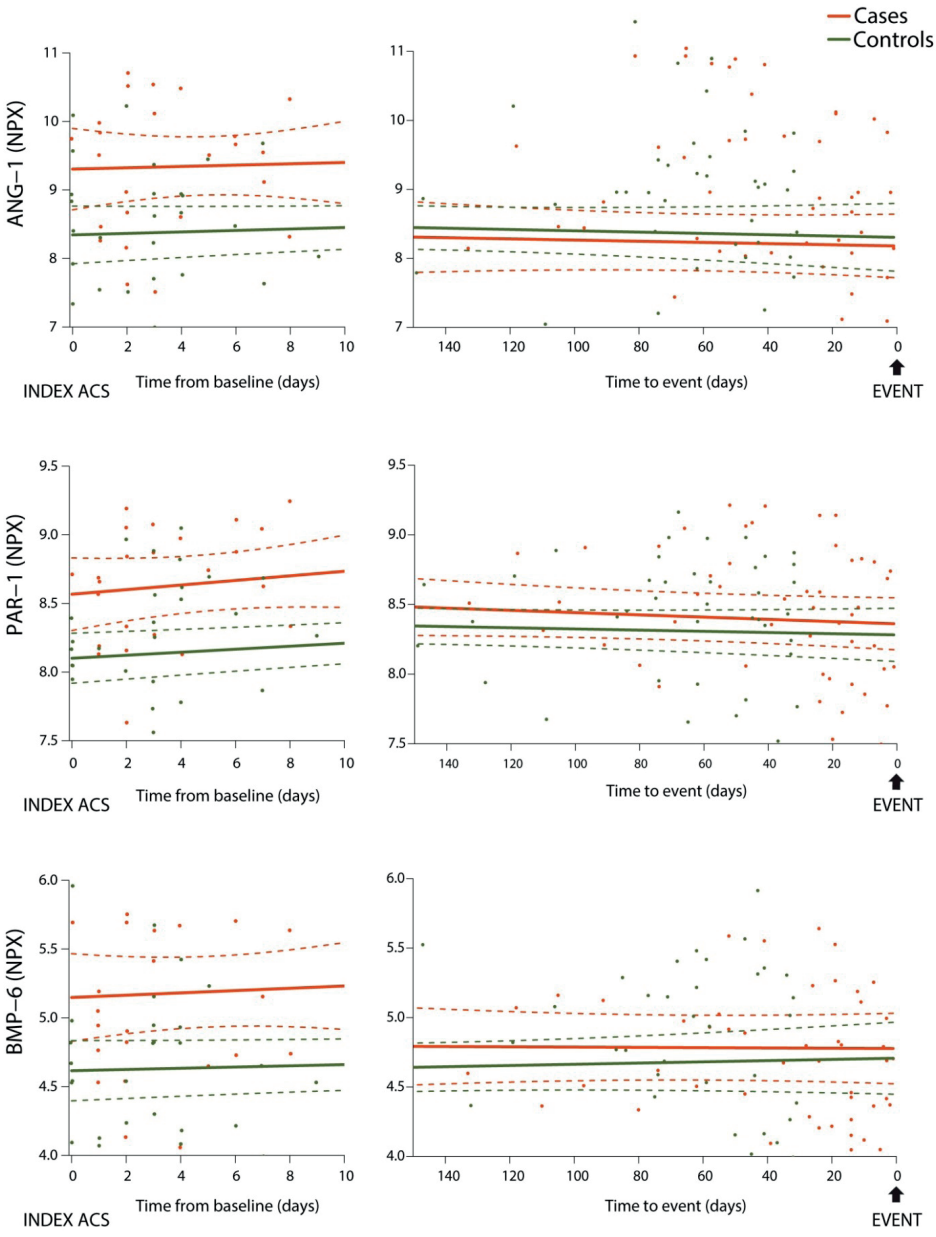

Figure 2. Time-course of PAR-1, BMP-6 and ANG-1
NPX: Normalized Protein eXpression

PAR-1 is a receptor expressed by cardiomyocytes, fibroblasts, smooth muscle cells and vascular endothelial wall cells.[8] Basic scientific research showed that PAR-1 may stimulate pathological remodeling after cardiac ischemia/reperfusion injury.[8, 9] More-

Table 4. Biomarker serum levels in the first 30 days for cases only

Biomarker (NPX)	Early cases[a]	Late cases[a]	p-value
NEMO	6.61±1.08	5.80±1.25	0.062
HB-EGF	5.62±0.89	5.03±0.73	0.049
SCF	8.31±0.87	8.08±0.60	0.40
PDGF subunit B	10.47±0.84	9.49±1.16	0.012
GDF-2	3.16±0.55	2.94±0.37	0.19
ANG-1	9.31±0.91	8.38±1.21	0.022
CCL3	4.04±0.59	3.59±0.36	0.013
TIE2	7.02±0.19	6.86±0.39	0.16
PAR-1	8.66±0.35	8.26±0.48	0.012
LEP	5.26±0.93	4.94±0.51	0.23
REN	8.36±1.34	8.31±1.06	0.91
TNFRSF11A	5.55±0.68	5.15±0.61	0.084
THPO	2.99±0.33	2.87±0.80	0.60
FGF-21	7.01±1.51	6.72±1.12	0.54
Gal-9	7.93±0.39	7.79±0.39	0.34
SRC	7.20±0.37	7.06±0.55	0.40
GH	8.02±2.14	7.27±1.96	0.31
XCL1	4.77±0.52	4.79±0.49	0.92
FGF-23	4.02±1.77	2.84±0.63	0.025
CCL17	8.74±1.14	8.57±1.26	0.70
IL-18	8.59±0.58	8.36±0.46	0.21
BMP-6	5.23±0.60	4.80±0.77	0.091
IL-6	6.00±1.86	4.89±1.37	0.062
AMBP	6.00±0.22	5.90±0.23	0.26
CD40-L	7.28±1.41	6.28±1.44	0.058

Blood samples ≤30 days after the index ACS were available for 15 early cases and 17 late cases.
* Patient-level mean value ± standard deviation.
NPX: Normalized Protein eXpression

over, PAR-1 is involved in hemostasis and thrombosis.[8, 10, 11] PAR-1 modulates thrombin signaling and is expressed on platelets, and may activate platelet secretion and aggregation. Local tissue injury of the vascular endothelial wall might induce endothelial responses via PAR-1, like recruitment of leukocytes and platelets, to manage infection or damage.[11] Accordingly, higher PAR-1 serum levels during the first 30 days following an ACS, might play a role in pathological remodeling after an ACS, and may lead to the development of (platelet-dependent) arterial thrombosis, and thus the recurrence of a coronary event.

BMP-6 is part of the transforming growth factor β family of cytokines. BMP-6 is involved in activation of osteogenic markers in mesenchymal stem cells, and may modulate ectopic cartilage and bone matrix formation.[12] Bone matrix formation is one of the

Table 5. Biomarker serum levels in relation to time post index ACS

	Median maximum value ≤7 days*		Patient-level mean value ≤30 days*		Patient-level mean value >30 days†	
Biomarker (NPX)	Cases	Controls	Cases	Controls	Cases	Controls
NEMO	6.50 (5.51-7.29)	5.75 (4.70-6.63)	6.18 ±1.22	5.67±1.33	5.44±1.58	5.66±1.50
HB-EGF	5.43 (4.73-5.82)	5.06 (4.48-5.60)	5.31±0.85	5.15±0.88	5.19±0.89	5.32±0.95
SCF	8.18 (7.45-8.88)	8.35 (7.99-8.77)	8.19±0.74	8.40±0.54	8.45±0.55	8.53±0.45
PDGF subunit B	10.66 (9.37-10.87)	10.39 (8.95-10.69)	9.95±1.12	9.72±1.20	9.37±1.45	9.72±1.34
GDF-2	3.33 (3.74-3.59)	3.26 (2.97-3.60)	3.04±0.47	3.28±0.41	3.43±0.48	3.49±0.42
ANG-1	9.51 (7.86-9.78)	8.75 (7.72-9.54)	8.81±1.17	8.52±1.16	8.20±1.25	8.51±1.19
CCL3	3.97 (3.48-4.22)	3.55 (3.21-4.12)	3.80±0.52	3.70±0.68	3.59±0.55	3.62±0.52
TIE2	7.01 (6.89-7.19)	6.88 (6.71-7.17)	6.93±0.32	6.93±0.31	7.01±0.27	6.96±0.32
PAR-1	8.69 (8.27-8.88)	8.36 (7.88-8.59)	8.45±0.47	8.25±0.51	8.40±0.48	8.37±0.51
LEP	5.09 (4.58-5.73)	5.00 (3.96-5.79)	5.09±0.74	4.90±1.22	5.06±0.86	4.96±1.09
REN	8.74 (7.21-9.88)	7.88 (7.05-8.75)	8.34±1.18	8.08±1.06	8.47±1.01	8.24±1.00
TNFRSF11A	5.44 (5.04-6.01)	5.10 (4.60-5.51)	5.34±0.67	5.10±0.69	5.22±0.66	5.09±0.59
THPO	3.01 (2.75-3.42)	2.66 (2.35-2.97)	2.93±0.62	2.72±0.48	2.70±0.65	2.67±0.44
FGF-21	7.33 (6.71-8.36)	6.36 (5.26-7.59)	6.86±1.31	6.60±1.51	7.14±1.21	6.48±1.70
GAL-9	7.94 (7.64-8.10)	7.78 (7.42-8.05)	7.86±0.39	7.79±0.45	7.86±0.37	7.84±0.40
SRC	7.31 (7.21-7.44)	7.34 (7.00-7.61)	7.13±0.47	7.02±0.83	6.77±0.99	6.92±0.93
GH	9.07 (6.46-9.76)	7.15 (5.93-8.92)	7.62±2.05	7.52±2.01	7.32±1.92	7.72±2.05
XCL1	4.72 (4.50-5.21)	4.49 (4.19-4.87)	4.78±0.50	4.53±0.55	4.73±0.57	4.60±0.55
FGF-23	3.32 (2.78-3.89)	2.80 (2.55-3.24)	4.02±1.77	2.93±0.71	3.18±0.81	3.03±0.70
CCL17	8.53 (8.21-9.64)	8.12 (7.57-8.97)	8.74±1.14	8.32±1.39	8.47±1.30	8.46±1.29
IL-18	8.67 (8.07-9.00)	8.50 (7.97-8.76)	8.59±0.58	8.44±0.59	8.37±0.41	8.50±0.56
BMP-6	4.96 (4.65-5.69)	4.61 (4.14-5.11)	5.23±0.60	4.68±0.59	4.77±0.69	4.67±0.62
IL-6	6.56 (4.42-7.18)	5.08 (4.24-5.75)	6.00±1.86	4.63±1.42	3.99±1.04	3.69±0.91
AMBP	5.99 (5.82-6.18)	5.94 (5.68-6.07)	5.99±0.22	5.87±0.24	5.97±0.22	5.89±0.22
CD40-L	7.01 (6.17-8.31)	6.31 (5.40-7.29)	7.28±1.41	6.75±1.49	6.11±1.62	6.37±1.66

Blood samples in the time windows 0-7, 8-30 and 30-365 days after the index ACS were available for 23, 32, 28 cases and for 44, 67, 70 controls.
*Median (25th-75th percentile) value of the patient-level maximum.
†Mean ± standard deviation value of the patient-level mean.
NPX: Normalized Protein eXpression

key processes responsible for vascular calcification.[13] Since BMPs are overexpressed in (vulnerable) atherosclerotic lesions, it is suggested that BMPs modulate vascular calcification.[14] Furthermore, it is observed that BMPs contribute to vascular inflammation.[14-16] Lastly, previous research has indicated that oxidative stress may induce BMP-6 expression and thereby vascular inflammation and calcification.[12] Thus, it could be hypothesized that post-ACS oxidative stress may induce higher BMP-6 serum levels, which – in turn - might induce vascular inflammation and a recurrent coronary event.

ANG-1 is a widely expressed biomarker and is involved in multiple cellular processes that occur following an ACS.[17-19] ANG-1 modulates endothelial cell survival, prolifera-

tion, migration and reorganization. Furthermore, it promotes angiogenesis and vascular quiescence. However, in the absence of vascular endothelial growth factor (VEGF) exposure, ANG-1 may promote vessel regression.[17] To the contrast of our study, previous research indicated that ANG-1 positively modulates cardiovascular disease, and promotes cardiomyocyte survival and reduces infarct size.[20-22] Furthermore, one study showed that a lower serum level of ANG-1 on admission date, significantly predicted the development of one-year major cardiovascular events in post-ACS patients.[22]

Despite the complexity of the process, it is of interest to study blood biomarkers that stimulate the intracellular MAPK-cascade, since they may serve as novel biomarkers for aggravation of CVD. Because of the exploratory character of this study, our study results are limited to the examination of divergent biomarker patterns and thus, primarily, are valuable for exploration and identification of (novel) protein blood biomarkers. Further research is needed to establish whether the studied protein blood biomarkers may actually be used to identify post-ACS patients who are at higher risk of developing a recurrent coronary event.

Limitations

Since we chose to use Olinks'Proteomics PEA high throughput analysis to efficiently analyze our samples for potential discovery of novel protein blood biomarkers, our study results lack generalizability because of the use of arbitrary units. In addition, although Olinks'Proteomics PEA is an assay that gives highly reproducible results.[23] PEA technique still needs improvements to assure complete reproducibility. Lastly, because of the small number of events in our study, differences between cases and controls may have been masked.

Conclusion

In conclusion, the serum levels of ANG-1, PAR-1 and BMP-6, all biomarkers that stimulate the MAPK-cascade, were significantly elevated in patients with ACS who developed an early recurrent coronary event. These signaling proteins warrant further study on their potential use as novel biomarkers to identify high risk post-ACS patients.

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Chapter 10

The temporal pattern of immune and inflammatory proteins prior to a recurrent coronary event in post-acute coronary syndrome patients

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ABSTRACT

Purpose

We assessed the temporal pattern of 29 immune and inflammatory proteins in post-acute coronary syndrome (ACS) patients, prior to the development of recurrent ACS.

Methods

High-frequency blood sampling was performed in 844 patients admitted for ACS during one-year follow-up. We conducted a case-control study on the 45 patients who experienced reACS (cases) and two matched event-free patients (controls) per case. Olink Proteomics' immunoassay was used to obtain serum levels of the 29 proteins, expressed in an arbitrary unit on the log2-scale (Normalized Protein eXpression[NPX]). Linear mixed-effects models were applied to examine the temporal pattern of the proteins, and to illustrate differences between cases and controls.

Results

Mean age was 66±12 years and 80% were men. Cases and controls had similar baseline clinical characteristics. During the first 30 days, and after multiple testing correction, cases had significantly higher serum levels of CXCL1 (difference of 1.00 NPX, p=0.002), CD84 (difference of 0.64 NPX, p=0.002) and TNFRSF10A (difference of 0.41 NPX, p<0.001) than controls. After 30 days, serum levels of all 29 proteins were similar in cases and controls. In particular, no increase was observed prior to reACS.

Conclusion

Among 29 immune and inflammatory proteins, CXCL1, CD84 and TNFRSF10A were associated with early reACS after initial ACS-admission.

INTRODUCTION

In the pathophysiology of atherosclerosis, the lipid metabolism and the immune and inflammatory systems are interconnected.[1, 2] It is known that both lipids and inflammatory biomarkers are affected by LDL lowering treatment, which importantly reduces the occurrence of cardiovascular events. However, despite adequately lowering LDL levels, a considerable number of patients with CVD will still develop adverse (coronary) events, especially those with a residual inflammatory risk.[3] Therefore, more insights in the role of the immune- and inflammatory systems are required.

The research field of proteomics offers a novel way to gain understanding of disease processes.[4] As the proteome is considered the end product of the genome, and has a regulatory role in all kinds of biological processes in the human body, proteins are fundamental to determine onset and progression of diseases, including CVD. The advantage of research on proteins is the direct information it may offer at tissue level, regardless of a patient's genotype. Novel technologies are emerging to simultaneously detect expression patterns of multiple proteins. These technologies offer the opportunity to assess expression patterns of proteins belonging to several pathophysiological pathways simultaneously.[5]

Studying the temporal behaviour of the proteome in patients with CVD prior to a recurrent coronary event may potentially lead to the identification of proteins related to progression of atherosclerosis. Therefore, we performed a controlled prospective study to assess the temporal pattern of a wide range of proteins involved in the immune- and inflammatory systems just prior to the recurrence of a coronary event during one year follow-up of patients admitted with an acute coronary syndrome (ACS).

METHODS

Study population

The 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) is a multicentre observational study with a design characterized by high-frequent blood sampling to assess the course over time of blood biomarkers during one-year follow-up of patients who have been admitted with an ACS, and to study the temporal pattern of these biomarkers just prior to the occurrence of an imminent repeat coronary event.[6] In brief, patients aged ≥40 years, who were admitted with an ACS, and who had ≥1 cardiovascular risk factor were eligible. The diagnosis ACS was based on typical ischemic chest pain, lasting >10 min in the preceding 24 h, in combination with objective evidence of myocardial necrosis, as obtained from the ECG (ST-segment elevation or dynamic ST-segment depression) or biochemistry (CKMB or cardiac troponin eleva-

tion). After enrolment, venepunctures were performed every two weeks during the first six months and every four weeks during the second six months of follow-up.

BIOMArCS was approved by the Institutional Review Boards of all 18 enrolling hospitals, and all participating patients provided written informed consent. BIOMArCS is registered in The Netherlands Trial Register NTR1698 and NTR1106.

Case-control design

For the current study, we applied a nested case-control design for protein measurements and statistical analysis. A total of 45 patients (cases) developed the primary study endpoint composed of cardiac death, non-fatal myocardial infarction (MI), or unstable angina (UA) requiring urgent coronary revascularization during one year of follow-up after the initial ACS. Each case was assigned to two controls matched on age, gender and admitted hospital. For reasons of efficiency, for each case, the blood sample at hospital admission and the last two samples prior to the recurrent endpoint event were selected. For matched controls, the blood sample at hospital admission and the blood sample that corresponds in time from enrolment with the timing of the case-event were selected.

We were interested in the temporal patterns of the proteins during the acute phase after the initial ACS (first 30 days), as well as during the stable phase after the initial ACS (30-day to 1-year time period). Thus, separate analyses were conducted for cases (and their matching controls) who experienced the event in the first 30 days of follow-up after the initial ACS, and for cases (and their matching controls) who experienced their event thereafter until one-year follow-up.

Protein measurements

Olink's high throughput Proximity Extension Assay (PEA) technique (Olink Proteomics AB, Uppsala, Sweden) was used to measure 29 immune and inflammatory proteins of the cardiovascular II panel (Table 1).[5] Detailed information on PEA Technique is available on Olink's website (www.olink.com). In brief, PEA technique allows for efficient quantification of multiple protein biomarkers simultaneously. Every measured protein is expressed in an arbitrary unit on the log2-scale called Normalized Protein eXpression (NPX). Hence, an *increase* or *decrease* of *one* NPX corresponds to a *doubling* or *halving* of the protein's serum level, respectively. To determine approximate serum concentrations, general calibrator curves are available on the website of Olink for each protein biomarker.

Statistical analysis

Continuous baseline characteristics are presented as medians with 25th and 75th percentile, and were compared between cases and controls with the Mann-Whitney U test.

Categorical baseline characteristics are presented as numbers with percentages and were compared between cases and controls with the Chi-square test.

Linear mixed-effects models were fitted for every protein (dependent). To allow individual variation per patient, random intercepts were included in the models. Likelihood

Table 1. Overview of the immune and inflammatory protein biomarkers

Abbreviation	Full name	Synonyms	Molecular function
ADAM-TS13	A disintegrin and metalloproteinase with thrombospondin motifs 13	C9orf8, vWF-CP	Hydrolase
ADM	Adrenomodullin	AM	Hormone
ACE2	Angiotensin I converting enzyme 2	peptidyl-dipeptidase A	Hydrolase/receptor
CXCL1	C-X-C motif chemokine ligand 1	GRO1, GROa, MGSA, FSP, NAP-3	Chemokine/growth factor
CEACAM8	Carcinoembryonic antigenrelated cell adhesion molecule 8	CGM6, CD66b	Protein binding
CTSL1	Cathepsin L1		Hydrolase
HO-1	Heme oxygenase (decycling) 1	HMOX1	Oxidoreductase
IL-1ra	Interleukin-1 receptor antagonist	IL1RN, IRAP, ICL-1RA	Cytokine
IL1RL2	Interleukin-1 receptor-like 2	IL1RRP2, IL36R	Receptor
IL-17D	Interleukin-17D		Cytokine
IL-27	Interleukin-27 subunit alpha and beta	IL27A, EBI3, IL27B	Cytokine
IL-4RA	Interleukin-4 receptor subunit alpha	IL4R	Receptor
LOX-1	Lectin-like oxidized LDL receptor 1	OLR1, CLEC8A	Receptor
LPL	Lipoprotein lipase	LIPD	Hydrolase
IgG Fc receptor II-b	Fc Fragment Of IgG, low affinity IIb receptor	FCGR2B, CD32B	Receptor
MARCO	Macrophage receptor with collagenous structure	SCARA2	Receptor
hOSCAR	Osteoclast associated, immunoglobulin-like receptor	OSCAR	Receptor
PTX3	Pentraxin 3	TSG14,TNFAIP5	Receptor
PIgR	Polymeric immunoglobulin receptor		Receptor
IL16	Pro-interleukin-16	LCF	Cytokine
PD-L2	Programmed cell death 1 ligand 2	PDCD1LG2, B7DC, CD273	Receptor
RAGE	Advanced glycosylation end product-specific receptor	AGER	Receptor
CD84	SLAM family member 5	SLAMF5	Receptor
SPON2	Spondin-2	Mindin, DIL1	Antigen binding
CD4	T-cell surface glycoprotein CD4		Antigen binding
TF	Coagulation factor III (tissue factor)	F3, thromboplastin	Receptor
TRAIL-R2	TNF-related apoptosis-inducing ligand receptor 2	TNFRSF10B,CD262, DR5	Receptor
TNFRSF10A	Tumor necrosis factor receptor superfamily member 10A	CD261, DR4, TRAILR-1, APO2	Receptor
TNFRSF13B	Tumor necrosis factor receptor superfamily member 13B	CD267, TACI	Receptor

ratio tests and F tests were used for hypothesis testing, and model assumptions were checked by visual examination of the residuals. To account for the 29 proteins tested, correction for multiple testing was applied (p=0.003).[7] The corrected significance level was calculated using the matrix spectral decomposition method, a correction method used in 'omics' studies to account for the number of independent performed tests.[7, 8] All statistical tests were two-tailed. R statistical software (version 3.4.0) was used for statistical analyses, in particular the package nlme (https://cran.r-project.org/web/packages/nlme/index.html).

RESULTS

Baseline characteristics

Mean age was 66 ±12 years and 80% were men. Presentation, initial treatment, cardiovascular risk factors and medication at first blood sample (baseline) were similar for cases and matched controls (Table 2). Thus, matching was successful.

Biomarker pattern within first 30 days post-ACS

15 Cases experienced a recurrent event within the first 30 days of follow-up. After correction for multiple testing, the serum level of CXCL1 in the first 30 days was 1.00 NPX (95% confidence interval [CI] 0.38-1.61) higher in cases than in their matching controls, which corresponds to a doubling of the CXCL1 serum level in cases. The serum levels of CD84 and TNFRSF10A were also significantly higher in cases than in their matching controls with a difference in these serum levels of 0.64 NPX (95%CI 0.25-1.03) and 0.41 NPX (95%CI 0.20-0.62), respectively. (Table 3, Figure 1 left-hand panel)

Biomarker pattern after 30 days

29 Cases experienced their recurrent event between 30 days and one year following their initial ACS. Prior to the recurrent coronary event in the 30-day to one-year period, serum levels of all studied protein biomarkers stabilised in cases and matched controls to indistinctive serum levels (Table 4). Hence, we found no significant divergent protein biomarker patterns between so-called late cases and matched controls. In particular, no (steady) increase was observed prior to the repeat event.

Since we did find significant divergent protein biomarker patterns between early cases and matched controls, we compared protein biomarker serum levels of ≤30-day cases with those of >30-day cases as a post-hoc analysis in the first 30 days post index-ACS (Table 5). Overall, most protein biomarker serum levels appeared to be higher in early cases.

Table 2. Baseline clinical characteristics of the patients

	Cases n=44	Controls n=87	p-value
Presentation and initial treatment			
Men	35 (79.5)	70 (80.5)	0.90
Age - yr	67.5 (57.3-77.5)	66.7 (57.4-75.5)	0.83
Admission diagnosis			0.38
STEMI	16 (36.4)	42 (48.3)	
NSTEMI	22 (50.0)	33 (37.9)	
UAP	6 (13.6)	12 (13.8)	
CAG performed	39 (88.6)	82 (94.3)	0.25
PCI performed	33 (86.8)	67 (81.7)	0.48
CKmax - U/L	418 (195-1142)	513 (169-1332)	0.94
Cardiovascular risk factors			
Smoking			0.81
Current	17 (38.6)	35 (40.2)	
Former	12 (27.3)	27 (31.0)	
Never	15 (34.1)	25 (28.7)	
Diabetes mellitus	16 (36.4)	32 (36.8)	0.96
Hypertension	21 (47.7)	44 (50.6)	0.76
Hypercholesterolemia	19 (43.2)	46 (52.9)	0.30
Creatinine - μmol/L	88 (73-93)	81 (67-97)	0.15
Cardiovascular history			
Peripheral arterial disease	10 (22.7)	7 (8.0)	0.018
Myocardial infarction	14 (31.8)	33 (37.9)	0.49
PCI	14 (31.8)	29 (33.3)	0.86
CABG	10 (22.7)	17 (19.5)	0.67
Stroke	9 (20.5)	5 (5.7)	0.010
Valvular heart disease	4 (9.1)	3 (3.4)	0.18
Heart failure	4(9.1)	1 (1.1)	0.025
Medication at first blood sample moment >7 days after the index ACS *			
Aspirin	35 (92.1)	76 (92.7)	0.91
P2Y12 inhibitor	36 (94.7)	74 (90.2)	0.41
Vitamin K antagonist	7 (18.4)	8 (9.8)	0.18
Statin	35 (92.1)	79 (96.3)	0.32
Beta-blocker	36 (94.7)	69 (84.1)	0.10
ACE inhibitor or ARB	34 (89.5)	65 (79.3)	0.17

Continuous variables are presented as median (25th-75th percentile). Categorical variables are presented as number (percentage).

* The first blood sample >7 days was taken at a median (25th-75th percentile) of 24 (16-34) days after the index ACS.

ACE: angiotensin converting enzyme, ARB: angiotensin II receptor blocker, CABG: coronary artery bypass grafting, CKmax: maximum creatine kinase during the index admission, NSTEMI: non-ST-elevation myocardial infarction, PCI: percutaneous coronary intervention, STEMI: ST-elevation myocardial infarction, Troponin ax: maximum troponin value during the index admission, UAP: unstable angina pectoris, yr: years

Table 3. Difference in protein biomarker serum level between cases and controls ≤30 days

Protein (NPX)	Coefficient	95%CI	p-value
ADAM-TS13	0.079	(-0.093-0.25)	0.36
ADM	0.36	(0.015-0.70)	0.041
ACE2	0.69	(0.18-1.20)	0.009
CXCL1	1.00	(0.38-1.61)	0.002
CEACAM8	0.38	(-0.070-0.83)	0.096
CTSL1	0.21	(-0.10-0.52)	0.18
HO-1	0.14	(-0.19-0.47)	0.39
IL-1ra	0.11	(-0.49-0.71)	0.72
IL1RL2	0.046	(-0.22-0.31)	0.73
IL-17D	0.23	(0.029-0.43)	0.026
IL-27	0.36	(0.093-0.62)	0.009
IL-4RA	0.48	(0.16-0.80)	0.004
LOX-1	0.16	(-0.23-0.54)	0.42
LPL	-0.12	(-0.42-0.17)	0.40
IgG Fc receptor II-b	0.17	(-0.25-0.60)	0.42
MARCO	0.086	(-0.069-0.24)	0.27
hOSCAR	0.15	(-0.040-0.33)	0.12
PTX3	0.34	(-0.13-0.80)	0.15
PIgR	0.040	(-0.032-0.11)	0.27
IL16	0.21	(-0.14-0.56)	0.23
PD-L2	0.21	(-0.010-0.42)	0.061
RAGE	0.40	(0.12-0.67)	0.006
CD84	0.64	(0.25-1.03)	0.002
SPON2	0.14	(0.026-0.25)	0.017
CD4	0.19	(-0.024-0.41)	0.080
TF	0.14	(-0.049-0.33)	0.14
TRAIL-R2	0.29	(-0.041-0.62)	0.084
TNFRSF10A	0.41	(0.20-0.62)	0.0004
TNFRSF13B	0.096	(-0.23-0.43)	0.56

For every protein biomarker, the difference in serum level between cases and controls is expressed in a relative arbitrary unit on the log 2 scale. Thus, An increase or decrease of *one* NPX corresponds with a doubling or a halving of the protein serum level.
ACS: acute coronary syndrome, CI: confidence interval, NPX: Normalized Protein eXpression

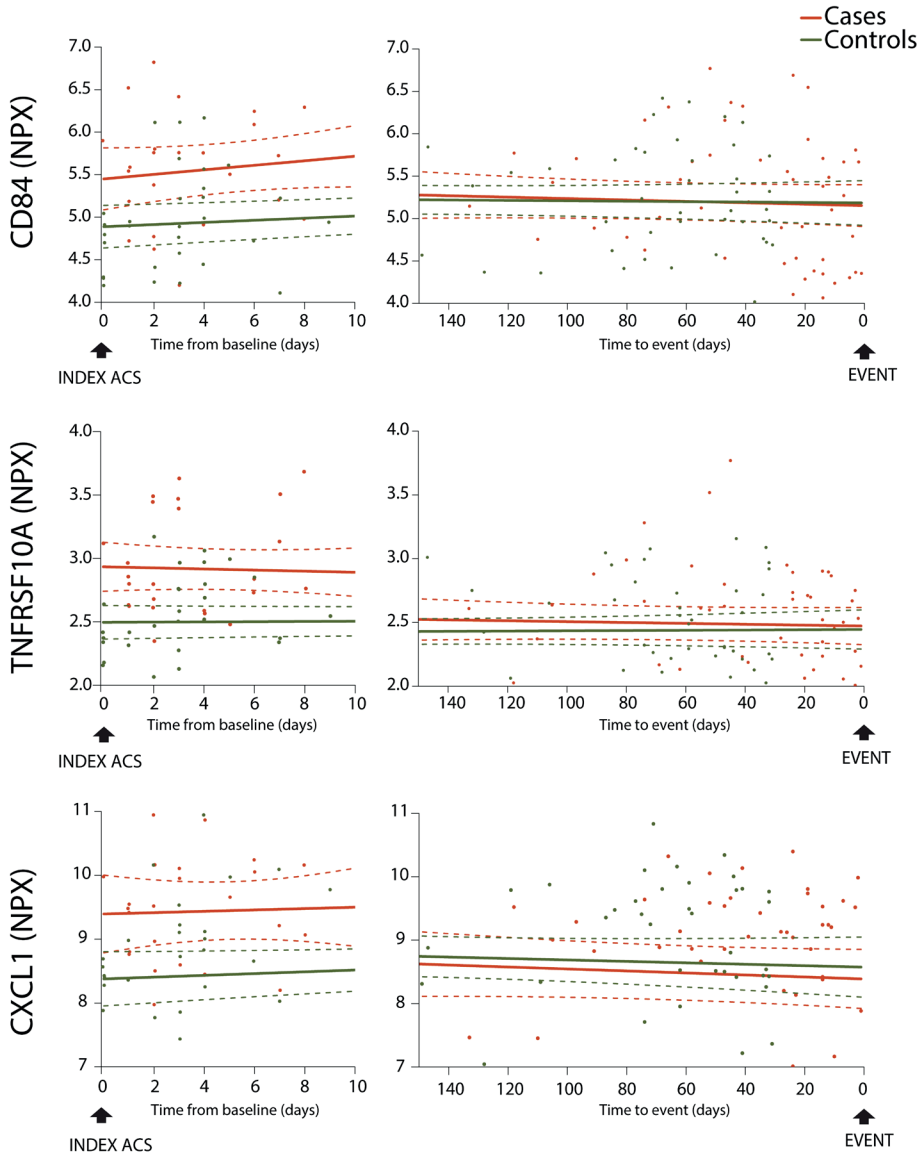

Figure 1. The temporal pattern of CD84, TNFRSF10A and CXCL1
NPX: Normalized Protein eXpression

Table 4. Protein biomarker serum levels in relation to time post index ACS

	Median maximum value ≤7 days*		Patient-level mean value ≤30 days*		Patient-level mean value >30 days†	
Protein (NPX)	Cases	Controls	Cases	Controls	Cases	Controls
ADAM-TS13	5.22 (5.02-5.47)	5.19 (4.94-5.31)	5.17±0.32	5.19±0.27	5.20±0.30	5.26±0.31
ADM	7.53 (7.10-8.01)	7.21 (6.75-7.54)	7.36±0.50	7.06±0.55	7.18±0.54	6.99±0.55
ACE2	4.24 (3.95-5.06)	3.95 (3.56-4.29)	4.27±0.92	4.00±0.79	4.21±0.62	4.18±0.86
CXCL1	9.42 (8.76-9.98)	8.68 (7.86-9.22)	8.95±1.07	8.67±1.22	8.46±1.29	8.76±1.22
CEACAM8	3.98 (3.52-4.25)	3.64 (3.09-4.12)	3.76±0.79	3.54±0.65	3.35±0.61	3.30±0.61
CTSL1	5.39 (5.06-5.57)	5.17 (4.85-5.48)	5.30±0.59	5.15±0.49	5.07±0.44	5.02±0.50
HO-1	13.00 (12.63-13.25)	12.79 (12.26-13.20)	12.71±0.50	12.77±0.54	12.55±0.34	12.77±0.52
IL-1ra	7.56 (6.60-8.07)	6.94 (6.44-7.52)	7.11±0.78	7.04±0.92	6.75±0.51	6.81±0.78
IL1RL2	3.13 (2.67-3.47)	3.07 (2.62-3.30)	3.07±0.41	3.07±0.43	3.14±0.34	3.07±0.44
IL-17D	2.49 (2.34-2.71)	2.18 (2.00-2.53)	2.34±0.35	2.22±0.31	2.26±0.22	2.25±0.36
IL-27	3.20 (2.69-3.55)	2.80 (2.51-3.13)	3.05±0.55	2.85±0.42	2.87±0.35	2.79±0.29
IL-4RA	2.21 (1.84-2.91)	1.86 (1.71-2.16)	2.22±0.66	1.88±0.30	1.81±0.26	1.82±0.32
LOX-1	6.33 (5.70-6.96)	5.99 (5.55-6.50)	6.08±0.69	6.03±0.62	5.85±0.49	5.92±0.50
LPL	9.26 (8.67-9.73)	9.09 (8.89-9.67)	9.23±0.50	9.30±0.52	9.40±0.53	9.34±0.49
IgG Fc receptor II-b	3.06 (2.62-3.50)	2.96 (2.43-3.39)	3.01±0.73	2.94±0.70	2.97±0.85	2.88±0.75
MARCO	5.00 (4.79-5.13)	4.93 (4.75-5.19)	4.98±0.22	4.98±0.29	4.99±0.26	5.02±0.29
hOSCAR	10.60 (10.42-10.78)	10.43 (10.19-10.77)	10.58±0.30	10.42±0.35	10.51±0.31	10.40±0.35
PTX3	3.96 (2.96-4.47)	3.38 (2.98-4.06)	3.43±0.82	3.31±0.68	3.03±0.56	2.91±0.51
PIgR	7.25 (7.10-7.33)	7.17 (7.07-7.27)	7.18±0.17	7.17±0.14	7.20±0.18	7.19±0.15
IL16	5.41 (5.18-5.82)	5.23 (4.85-5.54)	5.30±0.48	5.18±0.60	5.33±0.37	5.15±0.52
PD-L2	2.64 (2.30-3.06)	2.44 (2.16-2.74)	2.64±0.44	2.50±0.38	2.65±0.48	2.60±0.37
RAGE	5.57 (5.31-5.92)	5.30 (4.98-5.51)	5.45±0.50	5.14±0.39	5.30±0.51	5.13±0.43
CD84	5.54 (4.86-5.80)	4.93 (4.53-5.33)	5.34±0.62	5.09±0.63	5.17±0.67	5.24±0.65
SPON2	10.28 (10.11-10.43)	10.13 (9.95-10.26)	10.18±0.26	10.09±0.24	10.17±0.21	10.11±0.26
CD4	2.99 (2.48-3.25)	2.74 (2.45-3.02)	2.83±0.38	2.79±0.46	2.87±0.40	2.85±0.46
TF	5.60 (5.48-5.85)	5.55 (5.35-5.78)	5.55±0.31	5.54±0.32	5.59±0.37	5.58±0.36
TRAIL-R2	5.79 (5.48-6.42)	5.65 (5.34-5.93)	5.69±0.60	5.60±1.04	5.61±0.67	5.47±1.03
TNFRSF10A	2.80 (2.50-3.32)	2.48 (2.15-2.78)	2.69±0.44	2.46±0.40	2.50±0.39	2.45±0.36
TNFRSF13B	7.47 (7.33-7.86)	7.55 (7.05-7.85)	7.72±0.88	7.60±0.54	7.89±1.05	7.71±0.59

Blood samples in the time windows 0-7, 8-30 and 30-365 days after the index ACS were available for 23, 32, 28 cases and for 44, 67, 70 controls.

*Median (25th-75th percentile) value of the patient-level maximum.

†Mean ± standard deviation value of the patient-level mean.

NPX: Normalized Protein eXpression

Table 5. Protein biomarker serum levels in the first 30 days for cases only

Protein (NPX)	Early cases*	Late cases*	p-value
ADAM-TS13	5.25±0.30	5.10±0.34	0.20
ADM	7.53±0.50	7.21±0.45	0.067
ACE2	4.64±1.03	3.94±0.70	0.032
CXCL1	9.45±0.69	8.51±1.16	0.010
CEACAM8	3.98±0.72	3.56±0.82	0.13
CTSL1	5.42±0.59	5.20±0.59	0.30
HO-1	12.91±0.49	12.53±0.45	0.028
IL-1ra	7.23±0.84	7.00±0.73	0.42
IL1RL2	2.99±0.43	3.14±0.40	0.33
IL-17D	2.48±0.37	2.22±0.28	0.032
IL-27	3.26±0.59	2.87±0.45	0.045
IL-4RA	2.44±0.75	2.03±0.51	0.079
LOX-1	6.11±0.48	6.06±0.84	0.83
LPL	9.20±0.51	9.25±0.51	0.77
IgG Fc receptor II-b	2.97±0.74	3.05±0.74	0.77
MARCO	5.02±0.21	4.95±0.23	0.36
hOSCAR	10.62±0.23	10.54±0.35	0.48
PTX3	3.64±0.93	3.24±0.68	0.18
PIgR	7.23±0.11	7.14±0.20	0.10
IL16	5.34±0.42	5.27±0.54	0.68
PD-L2	2.70±0.35	2.58±0.51	0.45
RAGE	5.65±0.47	5.28±0.47	0.036
CD84	5.58±0.63	5.13±0.55	0.039
SPON2	10.28±0.21	10.08±0.27	0.031
CD4	2.95±0.33	2.72±0.39	0.079
TF	5.69±0.24	5.43±0.31	0.011
TRAIL-R2	5.88±0.64	5.53±0.53	0.10
TNFRSF10A	2.92±0.41	2.48±0.36	0.003
TNFRSF13B	7.66±0.40	7.78±1.17	0.71

Blood samples ≤30 days after the index ACS were available for 15 early cases and 17 late cases.
* Patient-level mean value ± standard deviation.
NPX: Normalized Protein eXpression

DISCUSSION

This study assessed the temporal pattern of 29 immune and inflammatory proteins in post-ACS patients. Serum levels of CXCL1, CD84 and TNFRSF10A showed to be significantly higher In cases than in matched controls prior to their recurrent coronary event within 30 days after the index ACS. After the first 30 days of follow-up, none of the studied protein biomarkers had detectable divergent serum levels in cases and their matched controls.

CXCL1 is a cytokine that attracts neutrophils by chemotaxis and stimulates monocyte arrest.[9] Oxidized LDL and wall shear stress on endothelial cells have been shown to induce the expression of CXCL1.[10, 11] Subsequently, CXCL1 stimulates monocyte adhesion to the endothelial wall.[12, 13] These monocytes migrate into the endothelial wall and stimulate the accumulation of macrophages. Eventually, this process promotes atherosclerotic plaque formation and instability and is therefore a key process in pathological atherosclerosis. Since we found higher serum levels of CXCL1 in early cases, a possible mechanism may be that CXCL1 is upregulated due to the index ACS, but subsequently also promotes early recurrent events by inducing atherosclerotic plaque instability.

CD84 is a signalling lymphocyte activation molecule (SLAM) and is expressed on platelets. It has been described that during thrombus formation, CD84 is triggered upon platelet aggregation and advances thrombus stability.[14] Since disproportional thrombus formation may cause arterial occlusion, CD84 may be of interest as a biomarker for coronary events. However, little research has been conducted on CD84 and its association with CVD. One previous study has identified CD84 to be associated with adverse outcome in Kawasaki disease coronary arteriopathy.[15] In this study, CD84 was found to be highly expressed in inflamed endothelium tissue of the coronary arteries of patients who developed adverse outcome and was suggested to play an important role in the pathogenesis of arterial thrombosis.[15] Our study found higher CD84 serum levels in post-ACS patients who developed early recurrent events. Potentially, CD84 upregulation is initiated by the index ACS and, subsequently, promotes disproportional thrombus formation causing early recurrent ACS. Further research should establish whether CD84 serum levels may be used to identify patients who will develop an early recurrent coronary event and who will not.

TNFRSF10A, a receptor for TNFSF10/TRAIL, is a member of the TNF-receptor superfamily and modulates apoptosis and proliferation of vascular smooth muscle cells.[16, 17] Since these processes may be beneficial as well as disadvantageous for atherosclerosis, depending on the stage of an atherosclerotic lesion, there is still an ongoing debate as to whether TNFRSF10A and its ligand may be used as a marker for progression or regression of atherosclerosis.[18] Our study found higher serum levels of TNFRSF10A in patients who developed an early recurrent cardiac event. Potentially, higher TNFSRF10A serum levels induce excessive proliferation of vascular smooth muscle cells after the index ACS which may lead to new coronary stenosis. [19]

Our study shows that CXCL1, CD84 and TNFRSF10A serum levels were elevated in post-ACS patients who experienced an early repeat coronary event. Nonetheless, we did not find divergent protein biomarker serum levels in post-ACS patients who experienced a late repeat coronary event (i.e. in the 30-day to one-year time-window). One may argue that differences in serum levels between cases and controls may be

smaller in the long-term and our study lacked power to reveal these. We designed the current study as an initial analysis and did not quantify all collected blood samples in BIOMArCS, since we intended - depending on the first results - to assess more blood samples after our first analysis to expand the number of repeated biomarker measurements. However, since we did find associations in early cases, and our study did not lack power to reveal these associations, we conclude that the 29 protein biomarkers we studied may not be associated with the development of recurrent coronary events in late cases. Apparently, the index ACS triggers short-term upregulation of CXCL1, CD84 and TNFRSF10A, which may play a role in the development of early recurrent coronary events.

For our protein measurements, we used Olink's PEA technique. This PEA technique enables an effective assessment of blood samples with a rapid high-throughput analysis of high sensitivity and specificity. However, although PEA is a promising technique, improvements are warranted to assure clinical valid and reproducible measurements. In addition to the technical challenges, one should consider that other factors related to biobank-sampling, i.e. blood sample collection and repeated freezing and thawing of collected blood samples influence the reproducibility of protein measurements. Studying the behaviour over time of immune and inflammatory proteins in patients with CVD prior to a (recurrent) coronary event may provide new insights on modulators of pathological atherosclerosis. However current research remains exploratory. Technical improvements are required to assess whether immune and inflammatory proteins can be used in clinical practice and may contribute to established clinical tools for disease detection and prognosis. Finally, the proposed mechanisms through which the biomarkers may be pathophysiologically related to the repeat ACS are hypothesis generating.

Conclusion

Among 29 immune and inflammatory proteins on the Olink platform, CXCL1, CD84 and TNFRSF10A were associated with early repeat coronary events in patients who experienced an ACS. Further research should assess whether CXCL1, CD84 and TNFRSF10A can actually be used to discriminate between patients who will experience an early repeat coronary event after an initial ACS admission, and patients who will not.

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Chapter 11

High-frequency metabolite profiling and the incidence of recurrent cardiac events in post-acute coronary syndrome patients

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ABSTRACT

Purpose

To study temporal changes in metabolite profiles in post-acute coronary syndrome (ACS) patients, in particular prior to the development of recurrent ACS (reACS).

Methods

BIOMArCS is a prospective study including patients admitted for ACS, who underwent high-frequency blood sampling during 1 year follow-up. Within BIOMArCS, we performed a nested case-cohort analysis of 158 patients (28 cases of reACS). We determined 151 metabolites by nuclear magnetic resonance in 7 (median) blood samples per patient. Temporal evolution of the metabolites and their relation with reACS was assessed by joint modelling. Results are reported as adjusted (for clinical factors) hazard ratios (aHR).

Results

Median age was 64 (25^{th}-75^{th} percentile 56-72) years and 78% were men. After multiple testing correction ($p<0.001$), high concentrations of extremely large VLDL particles (aHR 1.60/SD increase; 95%CI 1.25-2.08), very large VLDL particles (aHR 1.60/SD increase; 95%CI 1.25-2.08) and large VLDL particles (aHR 1.56/SD increase; 95%CI 1.22-2.05) were significantly associated with reACS. Moreover, these longitudinal particle concentrations showed a steady increase over time prior to reACS. Among the other metabolites, no significant associations were identified.

Conclusion

Post-ACS patients with persistent high concentrations of extremely large, very large and large VLDL particles have increased risk of reACS within 1 year.

INTRODUCTION

In recent years, the rise of genomics has helped to unravel the human genome and to identify genes that are involved with the development of CVD.[1] However, CVD is a polygenic and multifactorial disease, which is both influenced by a patient's genetic predisposition, as well as affected by biological and chemical variation downstream of the genetic code. Whereas genomic research concentrates on the 'static' genotype of a patient, metabolomic research focuses on metabolites, which are the substrates or end-products of all enzymatic processes.[2] Metabolomic research creates a blueprint of a patient's metabolism at a specific time point and, accordingly, captures both the upstream influence of a patient's genotype as well as downstream variation influencing the metabolism.[2] Eventually, combining knowledge gained through metabolomic research with knowledge on genetics and clinical risk factors, may give rise to novel insights on the pathophysiology of CVD.

The number of longitudinal studies that have assessed the association between a patient's metabolite profile and development of CVD is increasing.[3] However, these studies relate single baseline measurements to the incidence of CVD events during long-term follow-up.[3] Since the metabolite profile of CVD patients is not a static given, but will likely be influenced by changes in disease severity over time, repeated metabolite profile measurements might carry incremental prognostic information over a single measurement.

We designed the 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) to study temporal biomarker changes in post-acute coronary syndrome (ACS) patients. The current report describes an analysis of the temporal patterns of 151 metabolites in these patients and the association of the repeatedly measured metabolites with reACS.

METHODS

Study population

BIOMArCS is a multicenter observational study, conducted during 2008-2015 in the Netherlands. Details concerning the study design have been described elsewhere.[4] In brief, BIOMArCS enrolled patients who were admitted for ACS, either with or without ST-elevation, and who had at least one additional CVD risk factor. After inclusion, venipuncture was performed at admission, discharge, and subsequently every two weeks during the first half-year and every four weeks during the second half-year. If logistic circumstances hindered inclusion during hospitalisation, patients could be included on

the first outpatient visit within 6 weeks after discharge - the absence of early samples was then accepted. Samples were collected non-fasting.

BIOMArCS was approved by the Institutional Review Boards of all enrolling hospitals, and all participating patients provided written informed consent. BIOMArCS is registered in The Netherlands Trial Register as NTR1698 and NTR1106.

Study design

BIOMArCS enrolled 844 patients, and 45 reached the study endpoint of reACS, defined as a cardiac death, non-fatal myocardial infarction (MI) or unstable angina (UA) requiring urgent coronary revascularization (endpoint cases). For reasons of cost-efficiency, we applied a case-cohort design with respect to the present metabolite analysis. A random sample of 150 patients was selected from the full cohort (which rendered 8 endpoint cases), and was complemented with the remaining 37 endpoint cases outside this random sample. Consequently, the case-cohort sample included all 45 study endpoint cases and 142 endpoint-free patients.

We realized that the metabolites could have been influenced by the index ACS event. We were mainly interested in metabolite patterns after clinical stabilization. Therefore, we restricted our analyses to the 28 study endpoint cases and 130 event-free patients with available blood samples after 30 days following the index event.

Metabolite analysis

Serum samples were collected and preserved on-site at -80 degrees Celsius. Subsequently, samples were transported to the Erasmus MC for long-term storage under the same conditions. For the current analysis, serum samples were analyzed applying high-throughput automated proton NMR spectroscopy by Nightingale Health.[5] In each blood sample, all metabolites were quantified simultaneously, and, subsequently, expressed in absolute concentrations using Nightingale Health's proprietary software.[5] Details on the applied NMR method are described in the *supplemental material*. We assessed 151 metabolites, including 14 lipoprotein subclasses and their particle concentrations and lipids compositions, 9 cholesterol metabolites, 2 apolipoproteins, 8 glycerides and phospholipids, 9 fatty acids, 4 glycolysis related metabolites and 9 amino acids.

Statistical data analysis

Continuous variables are presented as median (25th-75th percentile). Categorical variables are presented as number (percentage). Differences in continuous data between study endpoint cases and event-free patients were evaluated by Mann-Whitney U tests, whereas categorical variables were evaluated by Pearson Chi-square tests.

The linear mixed effects (LME) model was used to describe the evolution of metabolites over time, with adjustment for age, gender, GRACE risk score, diabetes mellitus,

history of peripheral arterial disease, statin use and vitamin K antagonist use. Cox proportional hazard regression was used to relate serially measured metabolite level, based on the LME model, with the incidence of the study endpoint, while adjusting for GRACE risk. The parameters of the LME and Cox models were estimated in a joint model to avoid bias.[6] To enable a direct comparison of the relation between different metabolites and the study endpoint, we present adjusted hazard ratios (aHR) as per one standard deviation (SD) difference.

R statistical software (version 3.4.3) was used for the statistical analyses, in particular the package JMbayes.[6] All statistical tests were two-tailed, and p-values <0.001 were considered statistically significant, to correct for multiple testing. This significance level was determined by matrix spectral decomposition.[7]

RESULTS

Median (25^{th}-75^{th} percentile) age was 63.8 (56.1-71.6) years and 77.8% were men. Study endpoint cases were older than event-free patients, had a higher prevalence of diabetes, history of peripheral arterial disease and vitamin K antagonist usage (Table 1), and had similar characteristics otherwise. For the current analysis, a median (25^{th}-75^{th} percentile) of 7 [3-10] and 8 [5-9] repeated measurements were available in study endpoint cases and event-free patients, respectively.

In addition, 95% of the 1101 samples were collected in patients on statins. Clinical characteristics did not significantly differ between statin-treated and statin-untreated patients (data not shown). LDL cholesterol was 0.46 (95%CI: 0.061-0.85) mmol/l per SD increase higher in the 55 samples collected in statin-untreated patients (*p* value = 0.024).

Table 1. Baseline clinical characteristics

	Overall	Event-free patients	Cases	p-value
No. patients	158	130	28	
Presentation and initial treatment				
Men	123 (77.8)	102 (78.5)	21 (75.0)	0.88
Age - yr	63.8 (56.1-71.6)	62.3 (55.1-71.0)	68.0 (59.0-76.3)	0.030
Admission diagnosis				0.59
STEMI	69 (43.7)	59 (45.4)	10 (35.7)	
NSTEMI	66 (41.8)	52 (40.0)	14 (50.0)	
UAP	23 (14.6)	19 (14.6)	4 (14.3)	
CAG performed	149 (94.3)	121 (93.1)	28 100.0)	0.33
PCI performed	124 (84.4)	100 (83.3)	24 (88.9)	0.67
CKMax - U/L	425.0 (179.0-1197.0)	452.5 (196.8-1200.8)	312.0 (135.0-750.5)	0.24

Table 1. Baseline clinical characteristics (*continued*)

	Overall	Event-free patients	Cases	p-value
No. patients	158	130	28	
Cardiovascular risk factors				
Smoking				0.89
Current	65 (41.1)	54 (41.5)	11 (39.3)	
Former	48 (30.4)	40 (30.8)	8 (28.6)	
Never	45 (28.5)	36 (27.7)	9 (32.1)	
Diabetes mellitus	32 (20.3)	22 (16.9)	10 (35.7)	0.047
Hypertension	84 (53.2)	70 (53.8)	14 (50.0)	0.87
Hypercholesterolemia	76 (48.1)	66 (50.8)	10 (35.7)	0.22
Creatinine - µmol/L	82.5 (72.3-93.8)	82.0 (73.0-91.8)	86.5 (71.3-95.0)	0.46
Cardiovascular history				
Peripheral arterial disease	15 (9.5)	9 (6.9)	6 (21.4)	0.043
Myocardial infarction	51 (32.3)	42 (32.3)	9 (32.1)	1.00
PCI	47 (29.9)	37 (28.7)	10 (35.7)	0.61
CABG	16 (10.1)	11 (8.5)	5 (17.9)	0.25
Stroke	20 (12.7)	14 (10.8)	6 (21.4)	0.22
Valvular heart disease	5 (3.2)	2 (1.5)	3 (10.7)	0.055
Heart failure	7 (4.4)	4 (3.1)	3 (10.7)	0.20
*Medication at first blood sample moment >7 days after the index ACS**				
Aspirin	150 (95.5)	122 (94.6)	28 (100.0)	0.45
P2Y12 inhibitor	145 (92.4)	118 (91.5)	27 (96.4)	0.62
Vitamin K antagonist	14 (8.9)	8 (6.2)	6 (21.4)	0.028
Statin	151 (96.2)	125 (96.9)	26 (92.9)	0.64
Beta-blocker	135 (86.0)	108 (83.7)	27 (96.4)	0.15
ACE inhibitor or ARB	131 (83.4)	105 (81.4)	26 (92.9)	0.23

*The first blood sample >7 days was taken at a median (25th-75th percentile) of 24 (16-34) days after the index ACS.

Continuous variables are presented as median (25th-75th percentile). Categorical variables are presented as number (percentage).

ACE: angiotensin converting enzyme, ARB: angiotensin II receptor blocker, CABG: coronary artery bypass grafting, CKmax: maximum creatinine kinase during the index admission, No: Numero, NSTEMI: non-ST-elevation myocardial infarction, PCI: percutaneous coronary intervention, STEMI: ST-elevation myocardial infarction, Troponinmax: maximum troponin value during the index admission, UAP: unstable angina pectoris, yr: years

Metabolites

Higher concentrations of extremely large VLDL particles (XXL-VLDL-P), very large VLDL-P (XL-VLDL-P) and large VLDL-P (L-VLDL-P) were significantly associated with reACS (aHR 1.60/SD, 95% CI 1.25-2.08; aHR 1.60/SD, 95% CI 1.25-2.08; aHR 1.56/SD, 95% CI 1.22-2.05, respectively) during one year follow-up (Figure 1, *Supplemental Table S1*). Moreover, the concentrations of these particles steadily increased prior to the reACS (Figure 2).

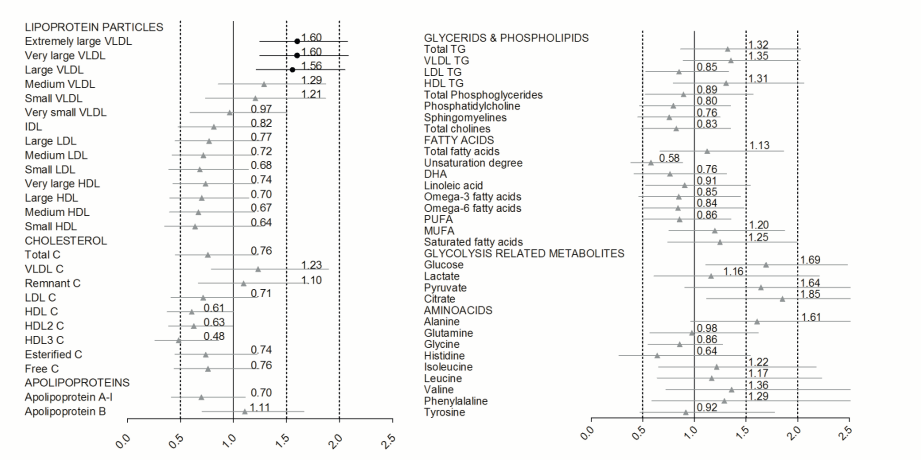

Figure 1. Associations of NMR-quantified metabolite profile and reACS

aHR's with 95%CI are presented on a SD-scale adjusted for age, gender, GRACE risk score, diabetes mellitus, history of peripheral arterial disease, statin use and vitamin K antagonist use. Black rounds are statistically significant with p<0.001, grey triangles are not.

C: cholesterol, DHA: Docosahexaenoic acid, HDL: high density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty, TG: triglycerides, VLDL: very low density lipoprotein

In addition to the lipoprotein subclass particle concentrations, the lipid composition of each lipoprotein subclass was quantified with NMR (*Supplemental table S2*). A lipoprotein particle is composed of phospholipids, cholesterol, cholesterol esters, free cholesterol and triglycerides. Figure 3 shows the aHR's of the lipid components of XXL-VLDL-P, XL-VLDL-P and L-VLDL-P. Overall, the individual lipid components of XXL-VLDL-P, XL-VLDL-P and L-VLDL-P were also associated with reACS. However, per lipid component we observed intra-variability (within the particle) and, more importantly, inter-variability (between the particles) in the degree of association with reACS. For instance, in XXL-VLDL the concentration of total cholesterol was associated with reACS with an aHR of 1.58/SD increase (95% CI: 1.18-1.94, $p < 0.001$). In XL-VLDL, the concentration of total cholesterol had an aHR of 1.53/SD increase (95%CI: 1.19-1.97, $p = 0.006$). In L-VLDL, the concentration of total cholesterol had an aHR of 1.34/SD increase (95% CI: 0.89-1.98, $p = 0.17$).

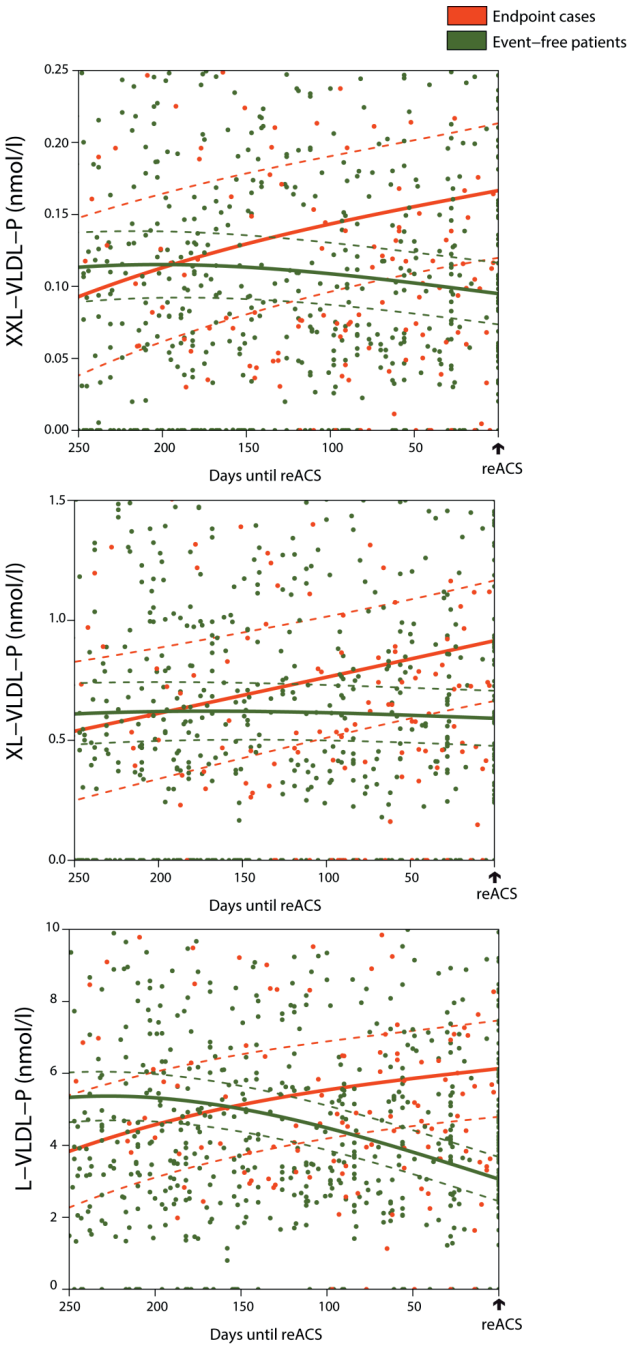

Figure 2. Longitudinal trajectory of XXL-VLDL-P, XL-VLDL-P and L-VLDL-P prior to reACS
nmol: nanomol, l: liter, L-VLDL-P: large VLDL particles, reACS: repeated acute coronary syndrome, XXL-VLDL-P: extremely large VLDL particles, XL-VLDL-P: very large VLDL particles

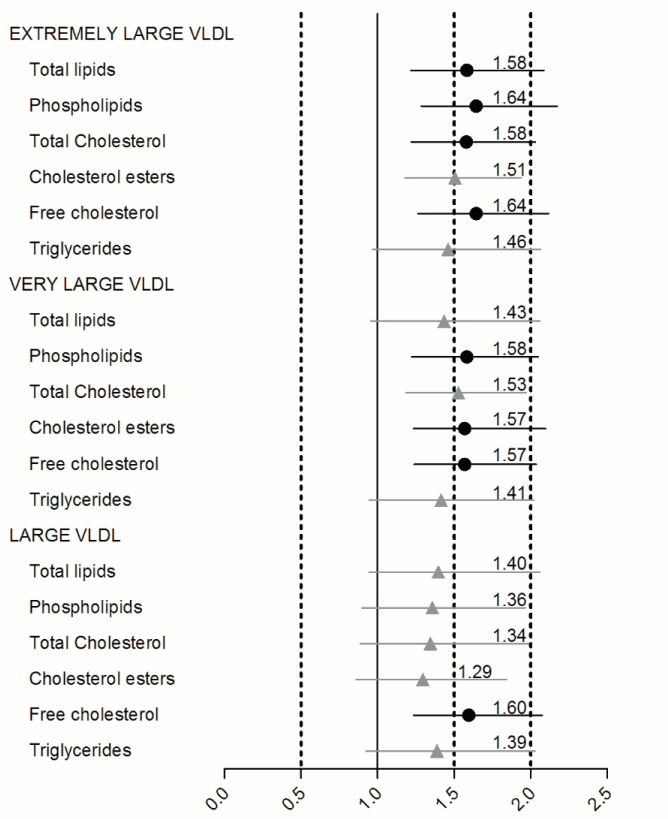

Figure 3. Associations of NMR-quantified components of extremely large, very large and large VLDL particles and reACS

aHR's with 95%CI are presented on a SD-scale adjusted for age, gender, GRACE risk score, diabetes mellitus, history of peripheral arterial disease, statin use and vitamin K antagonist use. Black rounds are statistically significant with p<0.001, grey triangles are not.

VLDL: very low density lipoprotein

Among the other assessed metabolite groups, no significant associations were identified between metabolite concentration and reACS.

DISCUSSION

This study assessed the association between repeatedly measured metabolite profiles and the incidence of reACS during one year follow-up in post-ACS patients. Patients who experienced reACS had steadily increasing concentrations of XXL-VLDL-P, XL-VLDL-P and L-VLDL-P during one-year of follow-up until the moment of the endpoint event. No significant associations were detected between longitudinal serum concentrations of cholesterol metabolites, apolipoproteins, glycerides and phospholipids, fatty acids,

glycolysis related metabolites or amino acids and reACS. Hence, serial blood sampling may benefit the prognostic accuracy of lipoprotein particle concentrations over a single baseline measurement. In a larger study cohort with more patients developing cardiac outcome, one should assess the frequency of sampling needed for accurate prognostication using lipoprotein particle concentrations.

Our study predominantly consisted of statin-treated patients. Previously, Würtz et al. showed in a combined analysis of population-based cohorts, that statin-use lowered most of their NMR-quantified metabolite concentrations.[8] In particular, statins effectively lowered multiple lipoprotein concentrations in addition to LDL cholesterol. In our study, despite statin use, XXL-VLDL-P, XL-VLDL-P and L-VLDL-P concentrations were significantly higher in patients who experienced a reACS, whereas total VLDL cholesterol was not. Since recent years, studies are advocating the added value of lipoprotein particle concentrations to lipoprotein cholesterol concentrations for clinical prognosis in patients with CVD.[9] Moreover, in 2011, the American National Lipid Association Expert panel has advised to study the use of lipoprotein particle concentrations to enhance treatment management, to address the residual risk of statin-treated patients with CVD for adverse outcome.[10] Subsequently, several studies have found that LDL particle concentration is a better predictor of adverse outcome than LDL cholesterol in CVD patients on lipid-lowering treatment.[9] One can argue that the latter finding might also be true for VLDL. It has been previously described that elevated VLDL cholesterol levels are an independent predictor of adverse outcome in the general population and in patients with CVD, and it has been suggested that VLDL cholesterol in combination with LDL cholesterol may be a better determinant of adverse outcome than LDL cholesterol alone.[11-13] In our current study, we found that the larger VLDL particle concentrations were associated with reACS, whereas total VLDL cholesterol was not. Hence, further research should establish if VLDL particle concentrations provide incremental prognostic information to LDL particle concentrations in statin-treated CVD patients to address their risk of developing adverse outcome.

Although not significant, plasma glucose appeared to correlate with reACS in our study. Previously, it has been demonstrated that hyperglycemia induces overproduction of larger VLDL particles.[14] Thus, potentially, the post-ACS patients who experienced reACS had a certain grade of hyperglycemia which may have induced the overproduction of larger VLDL particles and subsequent pathological atherogenesis.

Currently, results obtained by metabolite profiling are difficult to compare across various study populations, due to lack of a uniform way to quantify metabolites and otherwise heterogeneous study methods.[3] Although NMR is a cost-effective tool to obtain detailed knowledge on metabolites,[15] the sensitivity of this technique is limited compared with other metabolite profiling techniques such as mass spectrometry. Still, also mass spectrometry has downsides, including automation of the technique and the

fact that it cannot detect lipoproteins.[15] Therefore, in our view, NMR suits purposes of epidemiological studies including ours, whereas mass spectroscopy is more suited for detailed metabolite discovery. Eventually, the field of CVD metabolite research should focus on developing uniform study methods, as well as profiling techniques to obtain more reliable and comparable results. Under such conditions, the knowledge that will be gained through metabolite profiling might enable a precision-medicine approach to CVD treatment.

Limitations

The current study utilized 1101 serial blood samples, to assess the time course of NMR-quantified metabolites and their longitudinal association with incident ACS. Nonetheless, as only 28 study endpoint cases were available, we cannot exclude the possibility that our study was underpowered. In addition, freezing and thawing of serum samples could have influenced the metabolite measurements. However, our samples were kept frozen at -80 °C throughout complete storage and transportation of the samples up until quantification. Lastly, because of the exploratory character of our study, we could not provide a mechanical interpretation of our findings.

Conclusion

Post-ACS patients with persistent high concentrations of extremely large, very large and large VLDL particles have increased risk of reACS within 1 year.

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SUPPLEMENTARY APPENDIX

Method and validation of applied nuclear magnetic resonance technique

For our current study, samples were analyzed with Nightingale's nuclear magnetic resonance (NMR) metabolomics technology.[5] In brief, Samples were frozen at −80°C during long-term storage and kept frozen at −80°C throughout complete transportation of the samples to Nightingale's laboratory.

Samples were analyzed in 96-well plates containing 2 quality control samples per plate. The first quality control sample is a serum mimic, to ensure consistency of the quantifications. The second quality control sample is a mixture of 2 low-molecular-weight metabolites, for performance control of the automated liquid handler and spectrometer. NMR tubes were prepared with a mixture of 260 µL of serum and 260 µL of sodium phosphate buffer (75 mmol/L Na2 HPO4 in 80%/20% H2 O/D2 O, pH 7.4, including 0.08% sodium 3-(trimethylsilyl)propionate-2,2,3,3-d4 and 0.04% sodium azide). For the measurements, both the Bruker AVANCE III 500 MHx and Bruker AVANCE III HD 600 MHz spectrometers are applied. To ensure that samples remain cooled during the measuring process, the first spectrometer features a selective inverse room temperature probe head, and the later features a cryogenically cooled triple resonance probe head (CryoProbe Prodigy TCI). Fourier transformations to NMR spectra and automated phasing are executed by the computer systems of the Bruker AVANCE III 500 MHx and Bruker AVANCE III HD 600 MHz spectrometers. Subsequently, a central server executes quality inspection by controlling for background control, missing or extra peaks, baseline removal, and spectral area-specific signal alignments. In addition, each sample is compared with the two quality control samples to ensure the quality of the detected spectral information. All samples that suffice, are analyzed with regression modelling to obtain quantifications of the measured metabolites. Every individual metabolomic measure again has to pass multiple quality control steps and is compared with an extensive database of quantitative molecular data. Ultimately, the metabolite measurements that meet all pre-specified quality criteria are released. Nightingale's quality management system is certified by EN ISO 13485 and their NMR technique has been used so far for the analysis of over 500.000 samples for research purposes. A complete overview of the technique is described in Soininen et al. [5]

Supplemental Table S1. Metabolite associations with incidence of reACS

Metabolite	aHR(95%CI)	p-value
Lipoprotein particle concentrations		
Extremely large VLDL	1.60 (1.25-2.08)	<0.001
Very large VLDL	1.60 (1.25-2.08)	<0.001
Large VLDL	1.56 (1.21-2.05)	<0.001
Medium VLDL	1.29 (0.86-1.87)	0.21
Small VLDL	1.21 (0.74-1.87)	0.42
Very small VLDL	0.96 (0.59-1.51)	0.96
IDL	0.82 (0.48-1.31)	0.45
Large LDL	0.77 (0.45-1.27)	0.34
Medium LDL	0.72 (0.42-1.20)	0.21
Small LDL	0.68 (0.39-1.14)	0.13
Very large HDL	0.74 (0.43-1.20)	0.27
Large HDL	0.70 (0.40-1.15)	0.18
Medium HDL	0.67 (0.40-1.20)	0.16
Small HDL	0.64 (0.35-1.26)	0.18
Cholesterol		
Total C	0.76 (0.45-1.23)	0.28
VLDL C	1.23 (0.79-1.90)	0.35
Remnant C	1.10 (0.67-1.70)	0.64
LDL C	0.71 (0.41-1.22)	0.20
HDL C	0.61 (0.37-1.00)	0.054
HDL2 C	0.63 (0.39-0.99)	0.042
HDL3 C	0.48 (0.26-0.86)	0.010
Esterified C	0.74 (0.45-1.22)	0.25
Free C	0.76 (0.44-1.24)	0.31
Apolipoproteins		
ApoA1	0.70 (0.42-1.11)	0.15
ApoB	1.11 (0.70-1.66)	0.65
Glycerides & Phospholipids		
Total TG	1.32 (0.87-2.03)	0.20
VLDL TG	1.35 (0.89-2.02)	0.17
LDL TG	0.85 (0.53-1.33)	0.49
HDL TG	1.31 (0.80-2.06)	0.27
Total phosphoglycerides	0.89 (0.52-1.57)	0.66
phosphatidylcholine	0.80 (0.47-1.35)	0.42
Sphingomyelines	0.76 (0.45-1.25)	0.30
Total cholines	0.83 (0.48-1.35)	0.44
Fatty Acids		
Total fatty acids	1.13 (0.67-1.86)	0.64
Unsaturation degree	0.58 (0.39-0.89)	0.014
DHA	0.76 (0.42-1.31)	0.37

Supplemental Table S1. Metabolite associations with incidence of reACS (*continued*)

Metabolite	aHR(95%CI)	p-value
Linoleic acid	0.91 (0.53-1.54)	0.75
Omega-3 fatty acids	0.85 (0.46-1.44)	0.59
Omega-6 fatty acids	0.84 (0.50-1.48)	0.52
PUFA	0.86 (0.50-1.35)	0.56
MUFA	1.20 (0.75-1.87)	0.44
Saturated fatty acids	1.25 (0.74-1.99)	0.35
Glycolysis related metabolites		
Glucose	1.69 (1.11-2.48)	0.018
Lactate	1.16 (0.61-2.21)	0.66
Pyruvate	1.64 (0.91-2.98)	0.10
Citrate	1.85 (1.12-3.02)	0.014
Amino acids		
Alanine	1.61 (0.96-2.64)	0.068
Glutamine	0.98 (0.57-1.62)	0.98
Glycine	0.86 (0.55-1.27)	0.48
Histidine	0.64 (0.27-1.54)	0.36
Isoleucine	1.22 (0.65-2.18)	0.49
Leucine	1.17 (0.64-2.23)	0.61
Valine	1.36 (0.72-2.59)	0.35
Phenylalaline	1.29 (0.59-2.82)	0.52
Tyrosine	0.92 (0.47-1.78)	0.78

aHR's and 95%CI are presented on a SD-scale adjusted for: age, gender, GRACE risk score, diabetes mellitus, history of peripheral arterial disease, statin use and vitamin K antagonist use.
Apo: apolipoprotein, aHR: adjusted hazard ratio, C: cholesterol, confidence interval: CI, DHA: Docosahexaenoic acid, HDL: high density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TG: triglycerides, VLDL: very low density lipoprotein

Supplemental Table S2. Composition of lipoprotein particles and association with reACS

Lipid components	aHR (95%CI)	p-value	Lipid components	aHR (95%CI)	p-value
Extremely large VLDL			*Large LDL*		
Total lipids	1.58 (1.22-2.09)	<0.001	Total lipids	0.78 (0.45-1.29)	0.37
Phospholipids	1.64(1.29-2.17)	<0.001	Phospholipids	0.78 (0.46-1.33)	0.38
Total cholesterol	1.58 (1.22-2.03)	<0.001	Total cholesterol	0.77 (0.45-1.31)	0.36
Cholesterol esters	1.51(1.18-1.94)	0.008	Cholesterol esters	0.80 (0.45-1.31)	0.39
Free Cholesterol	1.64(1.26-2.12)	<0.001	Free Cholesterol	0.72 (0.44-1.17)	0.21
Triglycerides	1.46(0.97-2.06)	0.064	Triglycerides	0.83 (0.49-1.34)	0.47
Very large VLDL			*Medium LDL*		
Total lipids	1.43 (0.96-2.06)	0.086	Total lipids	0.71 (0.40-1.23)	0.22
Phospholipids	1.58 (1.22-2.05)	<0.001	Phospholipids	0.78 (0.44-1.37)	0.38
Total cholesterol	1.53 (1.19-1.97)	0.006	Total cholesterol	0.68 (0.37-1.16)	0.16
Cholesterol esters	1.57 (1.23-2.10)	<0.001	Cholesterol esters	0.69 (0.38-1.16)	0.18
Free Cholesterol	1.57 (1.24-2.04)	<0.001	Free Cholesterol	0.69 (0.41-1.13)	0.16
Triglycerides	1.41 (0.95-2.02)	0.088	Triglycerides	0.79 (0.46-1.34)	0.41
Large VLDL			*Small LDL*		
Total lipids	1.40 (0.94-2.06)	0.12	Total lipids	0.68 (0.38-1.13)	0.13
Phospholipids	1.36 (0.90-1.96)	0.15	Phospholipids	0.47 (0.28-0.84)	0.006
Total cholesterol	1.34 (0.89-1.98)	0.17	Total cholesterol	0.63 (0.36-1.10)	0.10
Cholesterol esters	1.29 (0.86-1.84)	0.21	Cholesterol esters	0.66 (0.37-1.12)	0.13
Free Cholesterol	1.60 (1.23-2.08)	0	Free Cholesterol	0.61 (0.36-1.05)	0.08
Triglycerides	1.39 (0.92-2.03)	0.10	Triglycerides	1.00 (0.60-1.64)	0.98
Medium VLDL			*Very large HDL*		
Total lipids	1.32 (0.87-1.97)	0.19	Total lipids	0.75 (0.46-1.23)	0.26
Phospholipids	1.30 (0.86-1.91)	0.22	Phospholipids	0.72 (0.41-1.13)	0.22
Total cholesterol	1.25 (0.82-1.84)	0.25	Total cholesterol	0.78 (0.45-1.28)	0.36
Cholesterol esters	1.18 (0.76-1.70)	0.40	Cholesterol esters	0.78 (0.48-1.39)	0.36
Free Cholesterol	1.29(0.86-1.87)	0.21	Free Cholesterol	0.73 (0.40-1.21)	0.26
Triglycerides	1.33 (0.88-1.98)	0.18	Triglycerides	0.81 (0.33-1.72)	0.72
Small VLDL			*Large HDL*		
Total lipids	1.23 (0.76-1.91)	0.39	Total lipids	0.70 (0.42-1.15)	0.13
Phospholipids	1.18 (0.72-1.84)	0.46	Phospholipids	0.71 (0.43-1.11)	0.16
Total cholesterol	1.08 (0.68-1.72)	0.73	Total cholesterol	0.71 (0.43-1.14)	0.17
Cholesterol esters	1.00 (0.62-1.64)	0.96	Cholesterol esters	0.70 (0.39-1.10)	0.16
Free Cholesterol	1.14 (0.71-1.78)	0.55	Free Cholesterol	0.69 (0.39-1.12)	0.16
Triglycerides	1.28 (0.83-1.94)	0.25	Triglycerides	0.76 (0.42-1.28)	0.34
Very small VLDL			*Medium HDL*		
Total lipids	0.94 (0.58-1.53)	0.83	Total lipids	0.65 (0.40-1.11)	0.10
Phospholipids	0.80 (0.48-1.36)	0.39	Phospholipids	0.62 (0.37-1.06)	0.080
Total cholesterol	0.94 (0.57-1.52)	0.82	Total cholesterol	0.69 (0.39-1.16)	0.15
Cholesterol esters	1.06 (0.64-1.71)	0.85	Cholesterol esters	0.70 (0.41-1.21)	0.22
Free Cholesterol	0.74 (0.40-1.24)	0.31	Free Cholesterol	0.62 (0.34-1.10)	0.090
Triglycerides	1.12 (0.70-1.68)	0.61	Triglycerides	1.61 (0.74-3.32)	0.31

Supplemental Table S2. Composition of lipoprotein particles and association with reACS (*continued*)

Lipid components	aHR (95%CI)	p-value	Lipid components	aHR (95%CI)	p-value
IDL			*Small HDL*		
Total lipids	0.81 (0.50-1.33)	0.41	Total lipids	0.62 (0.42-1.33)	0.12
Phospholipids	0.74 (0.44-1.21)	0.24	Phospholipids	0.79 (0.40-1.74)	0.57
Total cholesterol	0.80 (0.46-1.30)	0.41	Total cholesterol	0.56 (0.36-0.92)	0.030
Cholesterol esters	0.83 (0.46-1.40)	0.52	Cholesterol esters	0.53 (0.34-0.86)	0.016
Free Cholesterol	0.72 (0.43-1.17)	0.22	Free Cholesterol	0.66 (0.36-1.34)	0.19
Triglycerides	0.71 (0.40-1.25)	0.25	Triglycerides	1.36 (0.72-2.47)	0.32

aHR's and 95%CI are presented on a SD-scale adjusted for: age, gender, GRACE risk score, diabetes mellitus, history of peripheral arterial disease, statin use and vitamin K antagonist use.
aHR: adjusted hazard ratio, confidence interval: CI, HDL: high density lipoprotein, IDL: intermediate density lipoprotein, L: large, LDL: low density lipoprotein, reACS: recurrent acute coronary syndrome, VLDL: very low density lipoprotein.

Chapter 12

Variability of lipid measurements can have major impact on treatment during secondary prevention

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Submitted

INTRODUCTION

Hypercholesterolemia is one of the main modifiable risk factors for cardiovascular disease (CVD). The recently published update of the American (AHA/ACC) Cholesterol Clinical Practice Guideline provides recommendations regarding the use of lipid-lowering medication to reduce atherosclerotic cardiovascular risk.[1] This guideline advocates a treatment target for low-density lipoprotein cholesterol (LDL-C) to at least less than 70 mg/dl in very high CVD risk patients. In case of a clinical LDL-C measurement of more than 70mg/dl, adding ezetimibe and/or a PCSK9 inhibitor to a patient's maximally tolerated statin therapy has been indicated reasonable. The European (ESC/EAS/EACPR) guideline recommends similar treatment. [2]

Obviously, in clinical practice, the interpretation of a LDL-C measurement in relation to this treatment target is strongly related to the within-patient variability. Until now, the variability of cholesterol fractions has mostly been investigated in statin-naïve healthy volunteers. We studied the within-patient variability of total lipoprotein cholesterol, LDL-C and high-density lipoprotein cholesterol (HDL-C) in statin-treated patients after acute coronary syndrome (ACS), using longitudinal measurements during one year follow-up. We also determined reference change values (RCV), which reflect the range of biomarker (change) values that can still be explained by the combined within-patient and analytical variation.[3]

METHODS

We studied 157 patients post-ACS who participated in BIOMArCS[4,5] and were continuously treated with statins during one year of follow-up. Statin use was confirmed at every blood sampling moment. Per patient, we analyzed a median of 11 (range 3-19) samples (altogether 1783), which were collected in the 30-day to 1-year time-window after the index-ACS during which patients were clinically stable. Blood samples were analyzed batch-wise, and laboratory personnel were blinded for patient information. Total cholesterol and HDL-C levels were measured using the Beckman Coulter AU5811 (all with analytical coefficient of variation <5%). LDL-C levels were calculated using the Friedewald formula. Subsequently, within-patient variability of the cholesterol fractions was determined, as well as their corresponding RCVs.

RESULTS

Median age was 65 (interquartile rang 60-70) years and 80% were men. Median BMI was 27.2 (24.6-29.6) kg/m^2. 47% of the patients already used statins before first ACS admission. Median total cholesterol during follow-up was 159 (139-174) mg/dL, HDL-C 41 (36-46) mg/dL, and LDL-C 85 (73-100) mg/dL.

Within-patient variability was low: 5.9% for total cholesterol, 6.5% for HDL-C and 10.7% for LDL-C. Nevertheless, corresponding RCVs were considerable: 21.3% for total cholesterol, 22.7% for HDL-C and 32.7% for LDL-C. The distributions of cholesterol measurements per patient are shown in the Figure.

DISCUSSION

Our results show that in post-ACS patients using statins, total cholesterol, HDL-C, and LDL-C have low within-patient variability. Still, this variability can lead to seemingly clinically important differences between measurements during follow-up in individual subjects. For example, in a random patient with a habitual LDL-C of 70 mg/dL, taking into account the observed RCV, measurements that range between 47 mg/dL and 92 mg/dL may well be explained by natural and analytical variation. Obviously, these variations could lead to inappropriate reclassifications of the patient to being above or below the threshold, induce treatment adjustments, and, thus, cause over- or under-treatment.

We used up to 19 samples per patient to obtain within-patient variability of cholesterol fractions. However, we cannot exclude that the observed variability was influenced by the statin type and dosage, as these were not recorded. To minimize this possible influence, we only selected those patients that were continuously treated with the same statin type and dosage throughout follow-up.

In conclusion, although within-patient variability in cholesterol measurements seems relatively low in statin-treated patients, it can lead to clinically significant differences. Hence, applying guidelines based on targets may warrant critical consideration in clinical practice.

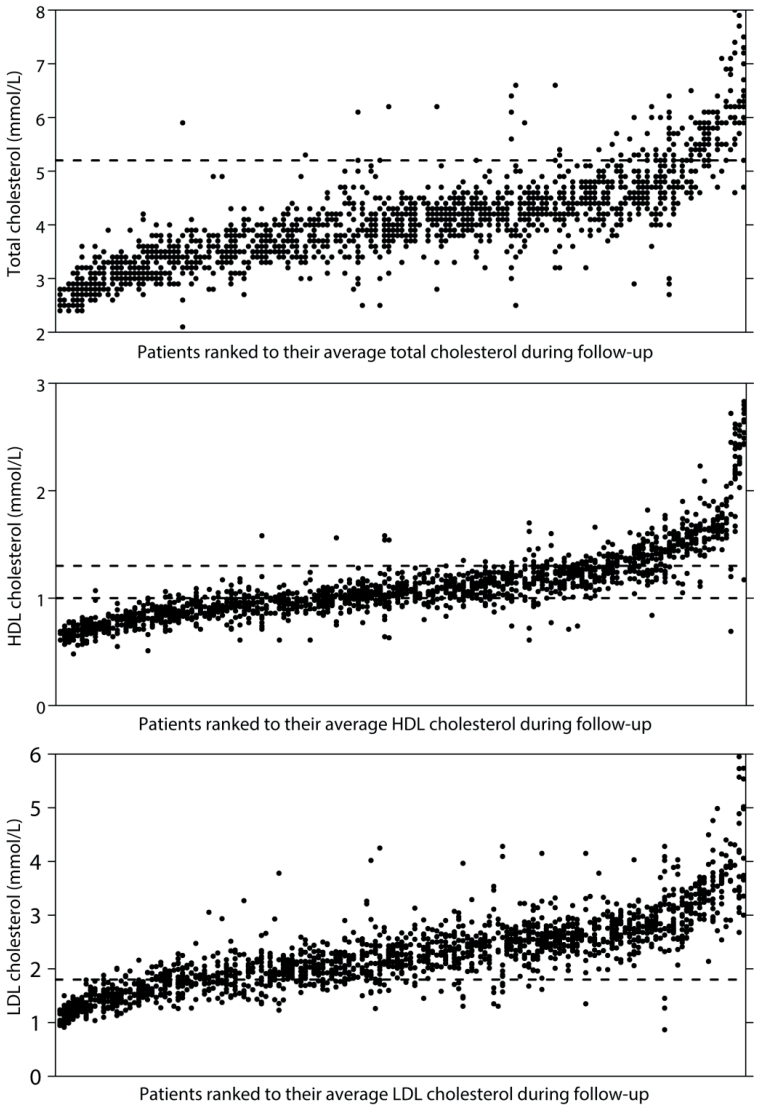

Figure. Distribution of cholesterol measurements per patient

Patients are ranked according to their mean biomarker level from left (lowest patient-specific mean) to right (highest patient-specific mean).

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Chapter 13

Anti-oxidized LDL antibodies and coronary artery disease: a systematic review

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Submitted

ABSTRACT

Background and aims

Antibodies to oxidized LDL (oxLDL) may be associated with improved outcomes in cardiovascular disease, however analysis is restricted by heterogenous study design and endpoints. Our objective was therefore conducting a comprehensive systematic review assessing anti-oxLDL antibodies in relation to coronary artery disease (CAD).

Methods

Through a systematic literature search, we identified all studies assessing the relationship of either IgG or IgM ox-LDL/ copper-oxLDL/ malondialdehyde-LDL with coronary atherosclerosis or cardiovascular events in populations with and without established CAD. Systematic review best practices were adhered to and study quality was assessed.

Results

Initial electronic database search identified 2,059 records, which was subsequently followed by abstract and full-text review. Finally, we included 18 studies with over 1,811 patients with CAD. Studes varied according to population studied, conventional cardiovascular risk factors and interventional modalities used to assess CAD. IgM anti-oxLDL antibodies were found to indicate protection from more severe CAD and possibly cardiovascular events, whilst the relationship with IgG is more complex and difficult to elucidate, with studies reporting divergent results.

Conclusion

In this systematic review there is evidence that suggests a relationship between anti-oxLDL antibodies and CAD, especially for the IgM subclass. However, further studies with well-characterised prospective cohorts will be important to clarify these associations.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death worldwide for the last 15 years,[1] despite significant pharmaceutical and technological advancements. There is therefore a clear mandate for better and earlier identification of patients at risk, as well as improved management of CVD when it occurs. Oxidized low density lipoprotein (oxLDL) is thought to be central to the atherosclerotic cascade, the common denominator in the pathophysiology of major adverse cardiovascular and cerebral events. OxLDL acts as an antigen which is recognised by macrophages and induces foam cell formation, with ensuing plaque lipid core development, apoptosis, cell death and cytokine production.[2] OxLDL is formed through the post-translational oxidative modification of LDL that has crossed the intimal arterial layer, becoming trapped beneath it in the sub-intimal space. OxLDL is actually a collective term reflecting a wide variety of oxidative changes to the LDL particle, including aldehyde adduction, such as malondialdehyde (MDA) onto Apolipoprotein B, the principle protein of LDL. Of note, post mortem studies have suggested that lesions with greater oxLDL deposition may be at increased risk of plaque rupture.[3, 4] Therefore, oxLDL is an ideal target for the investigation of potential identification of atherosclerotic plaques prone to rupture. Moreover, multiple studies have demonstrated that elevated levels of serum oxLDL are associated with the development of future CVD and often poorer prognosis, with conceivable clinical use as a biomarker.[5-12]

Moving on from the measurement of oxLDL, measurement of autoantibodies to oxLDL may allow improved cardiovascular risk stratification. Autoantibodies may be generated to any of the oxidation specific epitopes developed through the oxidative modification of LDL. Many studies have been performed to explore the association between autoantibodies to oxLDL and CVD, however, it is somewhat difficult to draw clear conclusions, given contradictory findings, variable study design with dissimilar endpoints, as well as different laboratory assays and techniques.

This systematic review aims to evaluate the studies that have been performed to assess the association between autoantibodies to oxLDL and cardiovascular mortality. There are autoantibodies to a wide variety of oxidation specific epitopes that have been evaluated in the literature, but this systematic review focuses on MDA-LDL, copper-oxidized LDL (Cu-oxLDL) and oxLDL (with non-specified oxidatively modified epitopes on LDL), the most widely reported autoantibodies. Additionally, given the contrasting endpoints used and the broad clinical spectrum covered by CVD, this review focuses on CAD with cardiac endpoints, including CAD severity as assessed at coronary angiography (CAG).

METHODS

Search strategy

In June 2018 we systematically searched Medline, Embase, Web of Science and Google Scholar electronic databases for relevant literature using different variations and abbreviations/ language variations of the following keywords: oxidized low-density lipoprotein, antibodies, autoantibodies, atherosclerosis, and coronary artery disease (detailed search strategy given in *Supplementary File 1*). In addition, we hand-searched the reference lists of relevant reviews in the field for identification of additional publications for inclusion. Our search was limited to peer-reviewed articles published in English and to studies on human adults. In addition, studies had to focus on healthy individuals or patients with CAD. Studies that centred primarily on patients with autoimmune diseases, for example Systemic Lupus Erythematosus, were excluded.

Review method and selection criteria

Studies were eligible either, if they reported an association between IgG and/or IgM autoantibodies to MDA-LDL, Cu-oxLDL, or total oxLDL and the occurrence of cardiovascular events during a follow-up of at least one year; or if they reported an association between IgG and/or IgM autoantibodies to MDA-LDL, Cu-oxLDL, or total oxLDL with degree of coronary atherosclerosis assessed by coronary angiography or other coronary imaging modality. We excluded cross-sectional studies that focused on differences in serum levels of MDA-LDL, Cu-oxLDL, or total oxLDL autoantibodies between patients with different types of CAD (myocardial infarction (MI), unstable angina, stable angina or prior MI), as the degree of CAD cannot be quantified using these definitions. In addition, studies were excluded that did not specify which subclass of autoantibody were evaluated (i.e. total versus IgG versus IgM), so as to permit analysis per immunoglobulin (Ig) subtype, given diverging associations with CVD reported in the literature.

Two physician reviewers (VJB and MMV) independently screened the publications for eligibility at title or abstract level. The remaining publications underwent full text review. Differences between reviewers regarding study selection were resolved by a third reviewer (RK). To assess the quality of the included studies we used the Newcastle-Ottawa Scale for cohort studies and case-control studies. For cross-sectional studies a modified scale was used.[13]

Data extraction

For each included study, data was extracted independently by two reviewers (VJB and MMV). Subsequently, the information was compared and merged, and discrepancies were resolved by consensus. The extracted data consisted of study and patient characteristics, autoantibodies of interest, method for determining the antibody levels, and

the association between the antibody levels and the clinical events or degree of CAD, as quantified by imaging. If studies used uni- and multi-variable models to assess these associations, we chose to include the results of the multivariable model adjusted for the most confounders.

RESULTS

The systematic literature search yielded a total of 2059 records potentially eligible for our current analysis. Based on titles and abstract, 1988 records were excluded, hence 71 studies underwent full text review. Subsequently, 53 studies were further excluded, as they did not meet the inclusion criteria. 18 original studies were included in the systematic review (*Figure 1*). Study populations and their baseline characteristics of the included studies are shown in *Table 1*. Results of the quality assessments for all studies are provided in *Supplementary Table 1*.

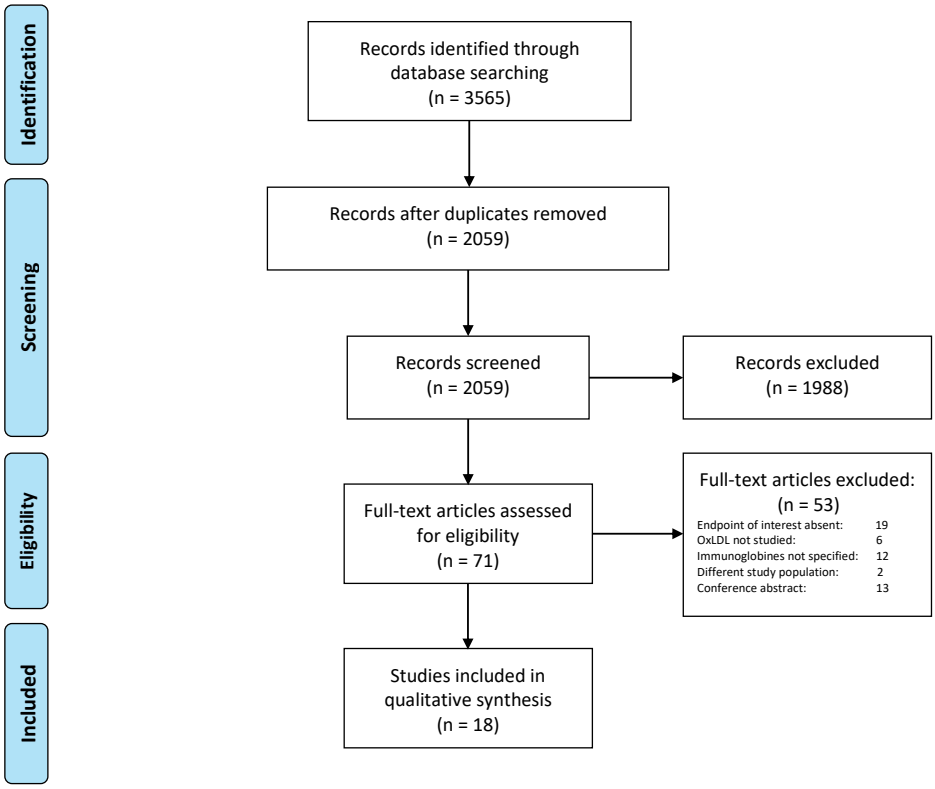

Figure 1. Flow diagram

Table 1 Baseline characteristics

Author	Year	Population (n)	Investigated biomarkers	Sample size		Age in years mean(SD)		Male gender (%)		DM (%)		HTN (%)		Current smoking (%)	
				CAD	no CAD	CAD	no CAD	CAD	no CAD	CAD	no CAD	CAD	no CAD	CAD	no CAD
Bilgen	2005	CAD patients (136). healthy controls (31)	IgG anti-oxLDL	136	31	57.6 (11.3)	53.5 (10.2)	70.5	67.8	13.2	0	30.9	0	47.8	0
Björkbacka	2016	Population-based prospective cohort (5393)	IgG-p45, IgG-p210, IgM-p45, IgM-p210	398	4995	61.1 [57.2-64.7]*	57.6 [52.2-62.5]*	61.6	39.8	19.8	7.4	81.2	62.5	33.8	26.1
Che	2011	CAG patients (154)	IgG anti-oxLDL	117	37	63.7 (10.6)	62.0 (11.5)	63.4	64.9	24.8	16.2	64.1	70.3	43.6	24.3
Chen	2011	CAG patients (558)	IgG anti-MDA-LDL, IgM anti-MDA-LDL	334	224	60.7	53.2	0	0	31.4	17.9	63.2	47.3	21.3	16.5
Garrido-Sanchez	2009	CAG patients (236)	IgG anti-oxLDL, IgM anti-oxLDL	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Gruzdeva	2014	STEMI patients (400), 33 healthy controls	IgG anti-oxLDL	400	NR	60.3 (1.1)	NR	67.5	NR	NR	NR	69.8	NR	42	NR
Khamis	2016	Hypertensive patients receiving blood-lowering treatment (1852)	IgG anti-MDA-LDL, IgM anti-MDA-LDL	485	1367	65.3 (7.8)	65.3 (7.6)	84.5	84.9	30.9	26.3	NR	NR	7.8	7.6
Maiolino	2012	CAG patients (733) b	IgG anti-MDA-LDL	733		63.3		78.4		NR		NR		7.3	
Meeuwsen	2017	168 endarterectomy patients	IgG anti-oxLDL, IgM anti-oxLDL	168		70.1 (9.6)		62.8		23.8		86.6		35.7	
Moohebati	2013	CAG patients (63), healthy controls (24)	IgG anti-oxLDL	31	56	59.4 (10.1)	58.3	38.7	58.9	41.9	10.7	64.5	44.6	51.6	25
Prasad	2017	Population-based prospective cohort (3509)	IgG anti-MDA-LDL, IgM anti-MDA-LDL	190	2914	43.7 (10.1)†		44.1†		11.6†		34.4†		29.3†	
Ravandi	2011	Population-based prospective cohort (2471)	IgG anti-MDA-LDL, IgM anti-MDA-LDL	748	1723	65.4 (7.8)	65.4 (7.8)	62.8	61.6	NR	NR	NR	NR	15.5	8.6
Rossi	2003	CAG patients (529)	IgG anti-MDA-LDL	445	84	63	62	NR	NR	NR	NR	NR	NR	NR	NR
Soto	2009	CAG patients (20), healthy controls (10)	IgG anti-oxLDL, IgM anti-oxLDL	13	17	59	36.5	100	52.9	23.1	0	69.2	11.8	46.2	11.8
Tsimikas	2007	CAG patients (504) a	IgG anti-MDA-LDL, IgM anti-MDA-LDL	504		60.1		61.7		NR		46.0		7.9	

Table 1 Baseline characteristics (*continued*)

Author	Year	Population (n)	Investigated biomarkers	Sample size		Age in years mean(SD)		Male gender (%)		DM (%)		HTN (%)		Current smoking (%)	
Tsimikas	2012	Population-based prospective cohort (765)	IgG anti-cu-oxLDL, IgM anti-MDA-LDL	138	627	68.8 (10.5)	61.4 (10.9)	48.5	59.4	13.8	6.9	75.4	66.0	17.4	20.4
van den Berg c	2018	143 CAG patients	IgG anti-MDA-LDL, IgM anti-MDA-LDL	143		59.6 (90)		84.6		19		62.7		28.7	
van den Berg c	2018	87 subjects with CHD, 227 subjects free of CHD	IgG anti-MDA-LDL, IgM anti-MDA-LDL	87	227	60.4 (6.3)	59.8 (6.4)	67.8	64.8	14.9	7.9	100	100	8	6.6
Wilson	2006	Population-based prospective cohort (2619)	IgG anti-MDA-LDL	151	2468	49.52†		NR	NR	NR	NR	NR	NR	NR	NR

If CAG patients were classified as having normal coronary arteries, there baseline characteristics are mentioned under 'without CAD'and combined with healthy controls if needed.
a: all patients were divided in either 2 groups. one with at least one stenosis with a diameter > 50% and one group without. As for the latter group. we cannot determine the baseline characteristics for the patients without any CAD. we chose to summarize all the baseline characteristics under known CAD.
b: of which 570 used for analysis
c: two separate cohorts are discussed in this paper, both are included separately in the systematic review
* median [IQR]
† Baseline characteristics only available for the entire group
CAD: coronary artery disease, CAG: coronary angiography, DM: diabetes mellitus, HTN: hypertension, MI: myocardial infarction, NR: not reported; SD: standard deviation

Autoantibodies against oxLDL and severity of CAD

A total of 11 cross-sectional studies were identified that explored the association between degree of CAD, as quantified by CAG, intravascular ultrasound (IVUS) or near-infrared spectroscopy (NIRS) and IgG oxLDL autoantibodies,[14-24] whilst five studies evaluated the relationship with IgM oxLDL autoantibodies.[16, 17, 22-24] Nine of these studies investigated all consecutive patients undergoing clinically indicated CAG,[14, 15, 17, 19-24] one study included only women undergoing CAG,[16] and one study included solely patients with ST-segment elevation MI (STEMI).[18] Endpoints were defined as number of diseased coronary arteries;[14, 17, 18, 21, 23] the Gensini score;[15] the Duke score[19] (both being composite scores for CAD lesion location and severity); a custom angiographic severity score;[16] or plaque characteristics determined by IVUS and lipid core burden index (LCBI) measured by NIRS, both measured in a non-culprit vessel.[24] Two studies divided their CAG patients in groups with at least one stenosis >50% and a group without stenosis >50%, and compared these two groups with healthy individuals.[20, 22] An overview of the study results is described in *Table 2*.

Except for Che et al.[15] and Gruzdeva et al.,[18] nine studies described a non-significant association between IgG anti-oxLDL antibodies or anti-MDA-LDL antibodies and the severity of CAD, as quantified at CAG.[14, 16, 17, 19-24] Che et al.[15] found a negative association between the natural logarithm of the (Gensini-score + 1) and serum IgG anti-oxLDL autoantibodies in 154 consecutive CAG patients. However, their findings seem to be heavily (negatively) biased by an outlying value of IgG and were not confirmed in multivariable analysis. In contrast to Che et al., Gruzdeva et al.[18] reported a positive association between IgG anti-oxLDL antibodies and the number of coronary arteries with a stenosis of >75% in patients undergoing CAG for STEMI. In a univariable logistic regression model with the dependent variable of one-vessel disease versus multivessel disease, IgG autoantibodies to oxLDL had a discriminative ability, expressed by a c-statistic of 0.85.

The association between IgM anti-MDA-LDL or total anti-oxLDL antibodies has been investigated less extensively. In the study by Garrido-Sanchez et al.[17] an inverse relationship between IgM anti-ox-LDL levels and the number of diseased coronary arteries was found ($p<0.005$). The same association was reported by Tsimikas et al.[23] for both IgM anti-MDA-LDL ($p = 0.027$) and IgM anti-Cu-oxLDL ($p = 0.030$). However, in a multivariable logistic regression model with the presence of obstructive CAD (defined as 1 or more stenosis of >50%) as the dependent variable, IgM anti-oxLDL level was not an independent predictor of obstructive CAD. Similarly, van den Berg et al.[24] reported that plaque burden or volume in a non-culprit vessel as determined by IVUS measurements was not significantly associated with IgM anti-oxLDL. In contrast, IgM anti-oxLDL was inversely associated with the degree of necrotic core in the same lesion and with the lipid core burden index (LCBI)-score of the worst 4mm in the measured segment.[24] The

Table 2. anti-oxLDL antibodies and Imaging

Author	Index group (n)	Reference group (n)	Statistical method	Confounders adjusted for in statistical analysis	Outcome	IgG anti-oxLDL	IgM anti-oxLDL
Bilgen	CAG patients (136)	Healthy controls (36)	ANOVA with post-hoc Tukey		Diseased coronary arteries (n)	NS	
Che	CAG patients (154)		Linear regression	hs-CRP, fasting glucose, serum albumin	ln(Gensini score + 1)	↓	
Chen	Female CAG patients (558)		Logistic regression	Age, smoking, and total and LDL cholesterol	<20% stenosis vs >20% stenosis	NS	↓
Garrido-Sanchez	CAG patiens (236)		NR		Diseased coronary arteries (n)	NS	↓
Gruzdeva	STEMI patients (400)	Healthy controls (33)	Kruskal-Wallis, followed by Mann-Whitney with Bonferonni correction		Diseased coronary arteries (>75%) (n)	↑	
Maiolino	CAG patients (733)		ANOVA		Duke CAD score	NS	
Moohebati	CAG patients (63)	Healthy controls (24)	ANOVA		1 or more stenosis (>50%) vs no stenosis vs healthy control	NS	
Rossi	CAG patients (529)		ANOVA		Diseased coronary arteries (>50%) (n)	NS	
Soto	CAG patients (20)	Healthy controls (10)	ANOVA		1 or more stenosis (>50%) vs no stenosis vs healthy control	unclear	↓
Tsimikas	CAG patients (504)		Logistic regression		Diseased coronary arteries (n)	NS	↓
van den Berg	CAG patients (143)		Linear regression	Age, gender, diabetes, smoking, previous statin use, LDL and HDL cholesterol	IVUS determined plaque characteristics in a non-culprit vessel, NIRS determined LCBI score in a non-culprit vessel	NS	↓

↑ Indicates a significantly possitive association with outcome, ↓ Indicates a significantly negative association with outcome.
CAD: coronary artery disease, CAG: coronary angiogram, hs-CRP: high-sensitive C-reactive protein, Ig: immunoglobulin, Ig: immunoglobulin, NS: Not significant, OxLDL: oxidzed LDL, STEMI: ST-elevation myocardial infarction

study by Chen et al.[16] also revealed a protective effect of IgM antibodies. In this study, patients with no to very mild (<20% stenosis) CAD had significantly higher IgM levels than patients with at least one stenosis of >20%, after adjusting for the effects of age, smoking, total cholesterol and LDL cholesterol. This inverse relationship seemed to be more profound in Caucasian women than in Afro-American women. However, when IgM anti-oxLDL serum levels were correlated with a custom-made CAD severity score that accounted for severity of stenosis, adjusted for collaterals and lesion location, no significant association was found. Finally, although the study by Soto et al.[22] did find higher IgM anti-oxLDL antibody levels in healthy controls and patients without significant CAD as quantified by CAG than in patients with CAD, these results should be interpreted with caution given only 30 patients were analysed (20 CAG patients and 10 controls).

Autoantibodies against oxLDL and cardiovascular events in patients without established CAD

We found four cohorts[5, 25-27] and three nested case-control studies[24, 28, 29] assessing the association between IgG and IgM anti-oxLDL and cardiovascular events in subjects without established CAD. There was significant variation in the frequency of cardiovascular risk factors present amongst the population-based studies; for example, Khamis et al. and Van den Berg et al. conducted their studies in subjects with hypertension.[24, 28] Study populations generally consisted mainly of Caucasians, whereas Prasad et al. included subjects differing in ethnicity (Caucasian, Black and Hispanic).[27]

All seven studies quantified autoantibodies in blood samples collected at baseline and assessed long-term cardiovascular outcomes. Björkbacka et al. distinguished additionally between amino acid sequences 661-680 (p45) and 3136-3155 (p210) of IgM and IgG.[25]

All seven studies assessed the association between IgG oxLDL autoantibodies and cardiovascular end points (*Table 3*). Both Tsimikas et al. and Prasad et al. found that elevated levels of IgG anti-oxLDL were associated with greater risk of developing future events (hazard ratio (HR) per standard deviation (SD) increase: 1.18, 95% confidence interval (CI) 1.03-1.37, and HR for fourth quartile vs first quartile: 1.97, 95%CI 1.30-2.99, respectively).[5, 27] Conversely, Khamis et al. found a protective association between IgG anti-oxLDL and cardiovascular end points, with cases having lower levels of IgG anti-oxLDL than controls (Odds ratio (OR) for third versus first tertile: 0.74, 95%CI 0.56-0.97).[28] The remaining four studies that assessed the association between IgG anti-oxLDL levels and cardiovascular end points did not detect significant associations.

Six studies assessed the association between IgM anti-oxLDL levels and cardiovascular end points (*Table 3*). Tsimikas et al. found that higher serum levels of IgM oxLDL autoantibodies were associated with a lower risk of developing cardiovascular end points (HR/SD increase: 0.69, 95%CI 0.50-0.95).[5] Van den Berg et al. also indicated a

Table 3. IgG and IgM Autoantibodies to Cu-oxLDL or MDA-LDL and cardiac end points in subjects without prevalent CAD

Author	study	Follow-up period, years	Statistical method	Matching variables	Confounders adjusted for in statistical analysis	Outcome	IgG anti-oxLDL	IgM anti-oxLDL
Björkbacka	Cohort study	> 15	Multivariabel Cox regression		Age, gender, LDL, HDL, SBP, triglycerides, hs-CRP, smoking, anti-hypertensive treatment, diabetes	Fatal and non-fatal MI, ischemic heart disease	NS (p45) NS (p210)	↓(p45) NS(p210)
Khamis	Nested case-control study	5.5 (median)	Conditional logistic regression	Age, Gender	Smoking, diabetes, SBP, total cholesterol, HDL, creatinine, BMI, family history of CAD, anti-hypertensive and statin treatment, CRP, NTproBNP	Fatal coronary heart disease, symptomatic non-fatal MI, coronary revascularisation, fatal and non-fatal stroke	↓	NS
Prasad	Cohort study	10.5 (median)	Multivariabel Cox regression		Age, gender, hypertension, diabetes, smoking, BMI, LDL, HDL, triglycerides	Cardiac death, non-fatal MI, stroke/TIA, unstable angina requiring hospitalisation and arterial vascularisation that included CABG, PCI, carotid endarterectomy, carotid stenting and peripheral artery revascularisation	↑	NS
Ravandi	Nested case-control study	6 (mean)	Conditional logistic regression	Age, gender, time of enrollment	Diabetes, smoking, SBP, LDL, HDL	cardiac death, hospital admission with CAD	NS	NS
Tsimikas (2012)	Cohort study	> 15	Multivariabel Cox regression		Age, Gender, previous CVD, SBP, smoking, diabetes, ferritin, LDL, HDL, alcohol consumption, social status, sport activity, CRP	Stroke,MI, new-onset unstable angina, acute coronary interventions and cardiac death	↑	↓
Van den Berg	Nested case-control study	4.5 (mean)	Conditional logistic regression	Age, gender, time of enrollment	Smoking, diabetes, baseline HDL, blood pressure treatments, either total IgG or total IgM.	fatal MI, non-fatal MI Q-wave criterium, non-fatal MI T-wave criterium, sudden death, new-onset ischemic heart disease or new-onset congestive heart failure	NS	↓
Wilson	Cohort study	> 8	Multivariabel Cox regression		Age, total cholesterol, HDL, smoking, SBP	Angina pectoris, unstable anginga pectoris, MI, cardiac death, TIA and stroke	NS	

Legend:

↑ Indicates a significantly higher level in the index group compared with the reference group, ↓ Indicates a significantly higher level in the index group compared with the reference group.

Ig: immunoglobulin, LDL: low density lipoprotein, MDA: malondiadehyde

strong protective effect of IgM anti-MDA-LDL antibodies on future CAD events (OR of third vs first tertile 0.29 (0.11, 0.76; p=0.012; p=0.016 for trend).[24] A similar protective association was seen in the study by Björkbacka et al. for IgM-p45 autoantibodies, with an adjusted HR for third vs first tertile of 0.59 (95%CI: 0.46-0.76; p<0.001). No significant association between IgM-p210 autoantibodies and cardiovascular end points was observed.[25] The remaining three studies did not find a significant association between IgM anti-MDA-LDL antibodies and events.[27-29]

Autoantibodies against oxLDL and cardiovascular events in patients with established CAD

Only two studies investigated the prognostic value of autoantibodies to oxLDL in CAD patients; one additional study was identified that included patients with known CVD (summarised in *Table 4*). The two CAD studies included (mainly Caucasian) patients undergoing clinically indicated CAG,[19, 23] and the CVD study included patients undergoing carotid endarterectomy.[30] Antibody levels determined from baseline samples taken prior to intervention were evaluated with events during follow-up. Among 168 patients undergoing carotid endarterectomy, Meeuwsen et al.[30] did not find any significant differences in baseline IgG and IgM anti-oxLDL in patients in whom the endpoint, a composite of cardiac death, non-fatal MI, stroke and percutaneous or peripheral intervention, later occurred and in whom it did not occur. Similar to the study by Meeuwsen et al., Tsimikas et al.[23] reported no significant associations between both IgG and IgM MDA-LDL autoantibodies levels and cardiovascular events in 504 patients included the BRUNECK study during a median follow-up of 4. However, during CAG, only 271 patients had obstructive CAD, the number of events (44; 20 all-cause deaths, 14 MIs, and 10 strokes) was low and the paper does not mention the statistical techniques used for analysis. On the contrary, Maiolino et al.[19] reported different results in patients undergoing clinically indicated CAG. In blood samples of 544 patients from the GENICA (Genetic and ENvironmental factors in Coronary Atherosclerosis) study, there was a significant positive association between IgG anti-MDA-LDL levels and both the occurrence of cardiovascular death (p = 0.04), and the occurrence of cardiovascular events (p = 0.005). In additional analysis, 136 of the 140 patients from the highest IgG anti-MDA-LDL quartile were successfully matched to 136 patients from the other three quartiles based on a propensity score that was computed with the most well-identified clinical characteristics associated with cardiovascular events. When comparing patients with high oxLDL antibody levels with their propensity matched controls, the patients with high levels had significantly less chance of cardiovascular death-free survival (83.1% vs 89.0%, p = 0.025) and less chance of cardiovascular event-free survival (69.2% vs 77.7%, p = 0.030), during a median follow-up of 7.2 years.

Table 4. IgG and IgM anti-oxidized LDL antibodies and cardiac end points in subjects undergoing clinically indicated CAG

Author	Index group (n)	Reference group (n)	Follow-up period, years	Statistical method	Matching variables	Confounders adjusted for in statistical analysis	Outcome	IgG anti-oxLDL	IgM anti-oxLDL
Maiolino	CAG patients from highest IgG anti-MDA-LDL quartile (136)	CAG patients from the lower three IgG anti-MDA-LDL quartiles matched based on propensity score	7.2 (median)	Kaplan-Meier	Gender, Age, BMI, LDL- and HDL-cholesterol, triglycerides, serum creatinine, homocysteine, glycemia, serum sodium concentration, heart rate arterial hypertension, smoking habit, LVEF, the Duke Prognostic Index of coronary athersosclerotic burden, length of follow-up, history and treatment variables		Cardiac death, composite of non-fatal MI, non-fatal stroke, and cardiac death	↑	
Meeuwsen	Carotid endarterectomy patients (168)		3	NR			Composite of cardiac death, stroke, non-fatal MI, coronary intervention, and peripheral intervention (including amputation)	NS	NS
Tsimikas (2007)	CAG patients (504)		4 (median)	NR			Composite of non-fatal MI, non-fatal stroke, and cardiac death	NS	NS

↑ Indicates a significantly higher level in the index group compared with the reference group, ↓ Indicates a significantly higher level in the index group compared with the reference group; NS: not significant.

BMI: body mass index, CAG: coronary angiography, HDL: high density lipoprotein, Ig: immunoglobulin, LDL: low density lipoprotein, LVEF: left ventricular ejection fraction, MDA: malondiadehyde, MI: myocardial infarction, oxLDL: oxidized LDL

DISCUSSION

This systematic review highlights that the results on associations between anti-oxLDL antibodies and cardiovascular endpoints described in the literature so far are heterogeneous and several aspects remain inconclusive. Moreover, despite a comprehensive search methodology, only 18 original studies were identified that satisfied the broad inclusion/ exclusion criteria. The studies identified were generally of a very high standard, as assessed by the (modified) Newcastle-Ottawa Scale, except for low scores reported for some of the cross-sectional studies (*Supplementary Table 1*). All of the included studies reported on IgG anti-oxLDL antibodies, with a smaller proportion reporting associations with IgM anti-oxLDL antibodies.

The studies investigating the relationship between anti-oxLDL antibodies and CAD as quantified by CAG (*Table 2*) varied in inclusion criteria and endpoint definition, and were often characterized by methodologically weak designs (*Supplementary Table 1*). Given the currently available evidence, there is little to support the hypothesis that IgG anti-oxLDL antibodies and CAD severity are related, except for perhaps in STEMI patients. IgM antibodies against oxLDL seem to have an inverse relationship with the CAD severity; however, it is unclear if this relationship is also as strongly apparent when adjusted for confounders. The inverse relationship between IgM antibodies and the necrotic core volume/ LCBI score, as demonstrated by van den Berg et al.,[24] is provoking and promising, as both endpoints have been shown previously to correlate with future cardiovascular events.[31] Future studies should use a well-validated score for CAD severity, use a regression analysis to establish the relationship between this (semi-) continuous endpoint and antibodies, as well as perform multivariable adjustments in order to confirm this relationship. In addition, there should be sufficient patients included to perform sub-group analysis based on CAG indication (e.g. STEMI, non-STEMI, stable CAD).

The seven studies (*Table 3*) conducted to assess the association of anti-oxLDL antibodies with cardiovascular endpoints in patients without objectified CAD demonstrated inconclusive conclusions with IgG anti-oxLDL antibody levels. Conversely, it seems that IgM anti-oxLDL levels are inversely associated with events. Moreover, the divergent results of the studies investigating cardiovascular endpoints with anti-oxLDL antibodies in patients undergoing clinically indicated CAG (*Table 4*) may be partially explained by differences in design, statistical analysis and number of events occurring. Current studies reported mixed endpoints, vastly different endpoints between studies, and the use of different experimental techniques or antibodies. Well-designed prospective studies with well-characterised populations, amongst diagnosed CAD patients or at-risk populations, will be needed to investigate further if anti-oxLDL antibody levels are indeed associated with cardiovascular events. It may also be prudent to focus on coronary heart disease

endpoints (i.e. non-fatal MI or cardiac death) as these are objective endpoints with more diagnostic certainty than their vascular or cerebrovascular counterparts. Naturally, this will require greater patient recruitment due to lower event rates; but may serve to more confidently assess potential associations. Another confounder to consider is the interaction of cardiovascular preventative medications. Statins have been demonstrated to somewhat counter-intuitively reduce IgM and increase IgG anti-MDA-LDL antibody levels, independent of dose.[32] Whereas, in the SARD (Standard versus high-dose therApy with Rosuvastatin for lipiD lowering) randomised clinical trial, rosuvastatin reduced total oxLDL levels in a dose-dependent manner. Thus, the interaction of statins with oxLDL is also complex and requires further clarification.

Despite the differences in study design and the presence of confounders discussed above, it appears that IgM anti-oxLDL antibodies indicate protection from CV events and more severe CAD, whilst the relationship with IgG is more complex and difficult to elucidate. The biological role of these autoantibodies needs to be considered, more than just their putative role as biomarkers in clinical practice. It is hypothesised that, as a key component of the innate immune system, IgM anti-oxLDL antibodies perform homeostatic functions, maintaining the equilibrium of atherosclerosis development. Perhaps in the presence of overwhelming stimulus, such as traditional cardiovascular risk factors or other inflammatory triggers, a mal-adaptive immune response occurs, with immunoglobulin class switching to IgG and accelerated atheroma deposition/plaque rupture.[2] Interestingly, a recent set of experiments using a single-chain variable fragment of E06, a naturally occurring IgM antibody that inhibits the uptake of oxLDL into macrophages, has demonstrated reduced levels of atheroma development, systemic inflammation and even prolonged life in LDL-receptor-null mice fed a high-cholesterol diet.[33] Thus, this study provides plausible mechanistic evidence for the theorised beneficial anti-inflammatory and anti-atherosclerotic actions of IgM anti-oxLDL antibodies.

Limitations

Our systematic review was mainly limited by the difficulty of comparability between studies in this particular research field. This was caused by several reasons. Firstly, laboratories are currently each using their own methods to measure and quantify the various anti- OxLDL antibody levels, since assays to measure these levels have not yet been standardised or commercialised. As a result, although we used broad inclusion criteria, anti- OxLDL antibody levels reported in the 18 included studies could not be directly compared. Secondly, although we focused on healthy populations or patient with CVD, and excluded patients with additional diseases such as auto-immune diseases, residual heterogeneity between the patient populations of the various included studies remained. Lastly, most included studies used (unstandardized) composite endpoints but failed to report the results for each individual endpoints.

Conclusion

Despite the paucity of studies and lack of conclusive data in the literature, this systematic review has highlighted clear signals of association between anti-oxLDL antibodies and CAD. IgM anti-oxLDL antibodies appear to indicate protection from more severe CAD and possibly cardiovascular events, whilst the relationship with IgG is more complex and difficult to elucidate. Further studies with well-characterised prospective cohorts will be important to clarify these associations.

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SUPPLEMENTARY APPENDIX

Search strategy

Database	# of refs	# of refs after de-duplication
Medline Ovid	867	193
embase.com	1365	1343
Web of science	1133	405
Google scholar	200	118
Total	3565	2059

12-06-2018

Medline Ovid

((oxidized low density lipoprotein.mp. AND (Antibodies/ OR Autoantibodies/ OR exp immunoglobulins/ OR exp Mast Cells/)) OR (((oxid* OR ox) ADJ6 (low-density-lipoprotein* OR ldl OR specific-epitope*) ADJ6 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR Igg* OR ige* OR igm* OR iga* OR mast-cell*)) OR ((oxLDL* OR ose) ADJ3 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR Igg* OR ige* OR igm* OR iga* OR mast-cell*))).ab,ti.) AND (exp arteriosclerosis/ OR (atherosclero* OR arteriosclero* OR athero-sclero* OR arterio-sclero* OR atherogenes* OR plaque* OR ((peripheral* OR coronar*) ADJ3 artery ADJ3 disease*) OR pad OR poad).ab,ti.) NOT (exp animals/ NOT humans/) AND english.la.

embase.com

('oxidized low density lipoprotein antibody'/exp OR (('oxidized low density lipoprotein'/de OR ('low density lipoprotein'/de AND (oxidation/de OR 'lipid oxidation'/de OR 'lipid peroxidation'/de))) AND ('immune response'/de OR antibody/de OR autoantibody/de OR 'antibody titer'/de OR 'antibody detection'/de OR 'immunoglobulin'/exp OR 'immunoglobulin antibody'/exp OR 'mast cell'/de)) OR (((oxid* OR ox) NEAR/6 (low-density-lipoprotein* OR ldl OR specific-epitope*) NEAR/6 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR Igg* OR ige* OR igm* OR iga* OR mast-cell*)) OR ((oxLDL* OR ose) NEAR/3 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR Igg* OR ige* OR igm* OR iga* OR mast-cell*))):ab,ti) AND ('atherosclerosis'/exp OR 'arteriosclerosis'/exp OR 'peripheral occlusive artery disease'/exp OR 'coronary artery disease'/exp OR (atherosclero* OR arteriosclero* OR athero-sclero* OR arterio-sclero* OR atherogenes* OR plaque* OR ((peripheral* OR coronar*) NEAR/3 artery NEAR/3 disease*) OR pad OR poad):ab,ti) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim

Web of science

TS=(((((oxid* OR ox) NEAR/5 (low-density-lipoprotein* OR ldl OR specific-epitope*) NEAR/5 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR lgg* OR ige* OR igm* OR iga* OR mast-cell*)) OR ((oxLDL* OR ose) NEAR/2 (antibod* OR anti OR autoantibod* OR Immune-respon* OR immunoglobulin* OR lgg* OR ige* OR igm* OR iga* OR mast-cell*)))) AND ((atherosclero* OR arteriosclero* OR athero-sclero* OR arterio-sclero* OR atherogenes* OR plaque* OR ((peripheral* OR coronar*) NEAR/2 artery NEAR/2 disease*) OR pad OR poad))) AND LA=(english)

Google scholar

“oxidized low-density-lipoprotein”|”ox ldl”|oxldl anti|antybodies|antybody atherosclerosis|arteriosclerosis|atherogenesis|plaque|”peripheral|coronary artery diseases|disease”

Supplemental Table 1. Quality control

	First author	Year	Selection				Comparability*	Outcome/Exposure			Total
cohort studies			*Representativeness of the exposed cohort*	*Selection of the non-exposed cohort*	*Ascertainment of exposure*	*Demonstration that outcome of interest was not present at start of study*	*Comparability of cohorts on the basis of the design or analysis*	*Assessment of outcome*	*Was follow-up long enough for outcomes to occur*	*adequacy of follow-up of cohorts*	number of stars awarded/ maximum number of stars
	Björkbacka	2016	*	NA	*		**	*	*	*	7/8
	Maiolino†	2013	*	NA	*	*	**	*	*		7/8
	Meeuwsen	2017	*	NA	*		**	*	*	*	7/8
	Prasad	2017	*	NA	*		**		*	*	6/8
	Tsimikas†	2007	*	NA	*	*			*		4/8
	Tsimikas	2012	*	NA	*		**	*	*	*	7/8
	Wilson	2006	*	NA	*	*	**	*	*	*	8/8
Case-control studies			*Case definition*	*Representativeness of the cases*	*Selection of controls*	*definition of controls*	*Comparability of cases and controls on the basis of the design or analysis*	*Ascertainment of exposure*	*same method of ascertainment for cases and controls*	*non-response rate*	number of stars awarded/ maximum number of stars
	Khamis	2016		*	*	*	**	*	NA	NA	6/7
	Ravendi	2011		*	*	*	**	*	NA	NA	6/7
	Van den Berg	2018		*	*	*	**	*	NA	NA	6/7

Supplemental Table 1. Quality control (*continued*)

	First author	Year	Selection				Comparability*	Outcome/Exposure		Total
Cross-sectional studies			*Representativeness of the sample*	*Sample size*	*Non-respondents*	*Ascertainment of the exposure (risk factor)*	*Comparability of subjects in different outcome groups*	*Assessment of the outcome*	*Statistical test*	number of stars awarded/ maximum number of stars
	Bilgen	2005	*		NA	**				3/9
	Che	2011	*		NA	**			*	4/9
	Chen	2011	*		NA	**	*			4/9
	Garrido-Sanchez	2009	*		NA					1/9
	Gruzdeva	2014	*		NA	**				3/9
	Maiolino†	2012	*		NA	**				3/9
	Moohebati	2014	*		NA	**				3/9
	Rossi	2003	*	*	NA	**	*	**		7/9
	Soto	2009	*		NA	**		*		4/9
	Tsimikas†	2007	*		NA	**	*		*	5/9

* Comparability can be rated with two stars

† Article applied both cohort design and cross-sectional design

Chapter 14

Summary and conclusions

This thesis focused on the identification of patients with cardiovascular disease (CVD), especially ischemic heart disease (IHD), who are at higher risk of recurrent CV events.[1, 2] Within the first year after hospital admission for IHD, mortality due to a recurrent CV event is 9%. [3] We hypothesized that a more precision-based, personalized approach to the course of IHD may improve prognostication, and, ultimately, secondary prevention.

Imaging of coronary atherosclerosis in patients with IHD

We have studied the extent of coronary atherosclerosis and coronary plaque morphology, as measured by invasive imaging modalities, including coronary angiography (CAG), intravascular ultrasound (IVUS) and near infra-red spectroscopy (NIRS), and assessed their relation with adverse clinical outcomes during long-term follow-up in patients admitted to the hospital with acute coronary syndrome (ACS) or stable angina pectoris (SAP).

The SYNTAX score is an established CAG-based tool to quantify the complexity of a patient's coronary artery disease (CAD), that incorporates luminal stenosis as well as other factors reflecting disease severity such as lesion length and disease diffusion. The SYNTAX score is limited to coronary luminography and therefore unable to qualify plaque morphology and vulnerability in itself. In **chapter two**, we studied 680 ACS/SAP patients, and revealed an association between coronary atherosclerotic complexity as measured by the SYNTAX score, based on all three coronary arteries, and coronary artery wall pathology as measured by IVUS and NIRS, which was obtained in a single non-culprit coronary artery segment.[4-6] In particular, SYNTAX score was associated with the amount of IVUS-derived fibrous/fibro-fatty tissue and NIRS-derived lipid burden. We found no association between SYNTAX score and the presence of thin-cap fibroatheroma (TCFA). As an important secondary finding, our study suggested that single-segment imaging might be sufficient to obtain an adequate assessment of a patient's coronary atherosclerotic status.

The SYNTAX score has been applied for prediction of adverse cardiac events up to five years after percutaneous intervention (PCI) and coronary artery bypass surgery (CABG).[7, 8] However, with regard to NIRS and IVUS, so far only their short-term prognostic value had been studied.[5, 9] We assessed the association between a range of NIRS- and IVUS-derived parameters, as measured by single-segment imaging in a non-culprit coronary vessel, and major adverse cardiac events (MACE) in ACS/SAP patients during four years of follow-up. In the 275 patients who had NIRS imaging, which we described in **chapter three**, each 100 unit increase of LCBI-derived maximum (in a 4 mm section) lipid core burden index (LCBI) was associated with a 19% increase in incidence of MACE. In the 581 patients who had IVUS imaging, which we described in **chapter four**, those with a lesion with a minimal luminal area ≤4.0 mm^2 had a 49% increase in MACE incidence. In addition, each 10 units increase in plaque burden

was on its own associated with a 26% increase in MACE incidence. Thus, in line with the result of **chapter two**, the extent and volume of coronary plaques as well as their lipid content, and not plaque features, appeared important factors for the prediction of adverse CV events. Additionally, we concluded that single-lesion imaging may be used for patient prognostication.

Finally, in **chapter five** we studied the long-term prognostic value of the SYNTAX score II (SSII). SSII incorporates both the original SYNTAX score as well as clinical factors, including age, gender, creatinine clearance, left ventricular ejection fraction, presence of peripheral vascular disease, chronic obstructive pulmonary disease, and unprotected left main CAD. SSII has been validated as a tool for prediction of long-term mortality in patients with complex CAD,[10-12] but had not yet been studied in a more heterogeneous population including less complex CAD. Hence, we examined its prognostic value in 628 ACS/SAP patients with baseline one- or two vessel disease, which is more common in routine clinical practice. In our study, each point increase in SSII was related with a 10% increase in all-cause mortality during 4.5 year follow-up, and its concordance index was 0.77 which can be classified as good performance. Thus, SSII may be used for prognostication purposes in CAD patients, irrespective of the extent and complexity of disease.

Circulating biomarkers of coronary atherosclerosis in patients with IHD

Circulating biomarkers may serve as a proxy for a patient's CVD progression and are therefore an appealing target for prognostic research in patients with IHD.[2] Currently, various biomarkers have been identified that might be associated with adverse CV outcomes in IHD patients. Interestingly, most research conducted on biomarkers uses single (baseline) measurements to predict adverse outcome, whereas the development of a CV event is a dynamic process. Surprisingly, little is known about the temporal evolvement of biomarkers identified in IHD. We postulated that such information is needed to successfully apply a biomarker-guided strategy for the identification and monitoring of high-risk IHD patients.

Hence, in **chapter six**, we assessed the temporal patterns of Myeloperoxidase and Galectin-3, two known markers of coronary plaque vulnerability, in 187 post-ACS patients prior to recurrent nonfatal or fatal ACS during 1 year of follow-up.[13, 14] Using repeated measurements, we studied the evolvement of both biomarkers early after ACS admission, as well as their temporal evolution prior to recurrent ACS. We found that Myeloperoxidase showed a peak of 78.3 ng/ml at the time of ACS admission, but decreased and stabilised within seven days post ACS admission. Thereafter, Myeloperoxidase remained stable throughout follow-up at an average level of 25.6 ng/ml (IQR: 20.4-32.0) and, notably, no increase in its level prior to recurrent ACS was detected.

Galectin-3 showed stable levels during the entire follow-up with an average level of 230 (167-300) pg/ml. Longitudinal levels of Myeloperoxidase and Galectin-3 were not associated with recurrent nonfatal or fatal ACS.

In **chapter seven** we studied the temporal pattern of Circulating Growth differentiation Factor-15 (GDF-15) during one year of follow-up in 111 post-ACS patients. GDF-15 is a stress-reactive inflammatory biomarker and has shown prognostic value in post-ACS patients.[15-17] In our study, GDF-15 level was systematically higher in the post-ACS patients who experienced recurrent nonfatal or fatal ACS, than in those who did not. After 30 days post the index-ACS, patients showed stable GDF-15 levels, which were on average 333 (95%CI 68-647) pg/ml higher in cases than in controls (1704 vs. 1371 pg/ml). Between-subject variability of GDF-15 was large (82% in cases and 84% in controls), and within-subject variability was low (18% in cases and 16% in controls). We concluded that GDF-15 may be used for prognostication in post-ACS patients, and individual reference values for GDF-15 would be preferred.

Chapter eight described the temporal pattern of soluble Suppression of Tumorigenicity-2 (sST2) during one year in 187 post-ACS patients. sST2 is an established marker of adverse cardiomyocyte remodelling after myocardial stress, and admission levels have shown to associate with adverse outcome in post-ACS patients.[18-20] Using repeated measurements, we found that persistently higher levels of sST2 during follow-up are associated with recurrent nonfatal or fatal ACS, as one standard deviation increase in sST2 level correlated with a 64% increase in incidence of recurrent CV events. Moreover, as we found that the within-patient variability over time in sST2 level is low, a limited number of measurements may be sufficient for prognostication.

In addition to these previously identified biomarkers of adverse outcome in IHD, we also studied a broad range of novel protein biomarkers in relation to recurrent CV events in post-ACS patients. For this analysis, Olink Proteomics' immunoassay was used to obtain the serum levels of these protein biomarkers, expressed in an arbitrary unit on the log2-scale (Normalized Protein eXpression[NPX]).[21] In **chapter nine** we described in 131 patients the temporal evolvement of 25 proteins that stimulate the mitogen-activated protein kinase (MAPK) cascade. The MAPK cascade is an intracellular cascade through which blood biomarkers modulate cell processes such as cell growth, differentiation or apoptosis.[22] In CVD development, the MAPK cascade has shown to be involved in plaque formation, stress-induced adverse myocardial remodelling and restenosis.[23] We found that the serum level of angiopoietin-1 (ANG-1), proteinase-activated receptor 1 (PAR-1) and bone morphogenetic protein 6 (BMP-6) was higher in post-ACS patients who experienced early repeat ACS within 30 days after their first ACS-admission. After 30 days, none of the 25 proteins were significantly higher in the patients who experienced recurrent ACS within one year. In the same patient group, we studied in **chapter ten** 29 immune and inflammatory proteins and

found that serum level of C-X-C motif chemokine 1 (CXCL1), slam-family member 5 (CD84) and tumor necrosis factor receptor superfamily member 10a (TNFRSF10A) was higher in the patients with repeat ACS within 30 days, but not after 30 days. Moreover, none of the exploratory proteins described in **chapter nine or ten** showed a steady or sudden increase in serum level prior to recurrent event.

In **chapter eleven** we continued to explore novel markers of repeat ACS in 158 post-ACS patients, studying (lipid) metabolites. For this analysis, high-throughput automated proton NMR spectroscopy was applied to simultaneously quantify 151 metabolites per serum sample.[24] Every standard deviation increase in concentrations of extremely large very low density lipoprotein (VLDL), very large VLDL and large VLDL particle concentration was respectively related to a 60%, 60% and 56% increased incidence of recurrent nonfatal or fatal ACS within one year post the index ACS. Notably, in the patients who developed recurrent ACS, the longitudinal particle concentrations of extremely large VLDL, very large VLDL and large VLDL increased steadily prior to recurrent ACS. Hence, repeatedly measuring these concentrations might provide relevant prognostic information in patients with IHD.

Lowering LDL cholesterol in patients with IHD effectively reduces their risk for recurrent CV events. The American and European guidelines recommend striving for LDL levels below 1.8 mmol/l in high-risk patients with CVD.[25, 26] However, so far the within-subject variability of LDL had been mainly studied in healthy individuals. Results of our analyses of within-subject variability in 157 statin-treated post-ACS patients, as presented in **chapter twelve**, showed a reference change value (RCV) for LDL of 32.7%. This RCV implies that a statin-treated patient with a habitual LDL level of 1.8 mmol/l may have clinical measurements that range between 1.2 mmol/l and 2.4 mmol/l that can be well explained by natural and analytical variation. As these variations may lead to inappropriate cholesterol treatment adjustments, the intra-subject variability of LDL should be considered while adjusting treatment in clinical practice.

Finally, in **chapter thirteen** we systematically assessed the current available literature on the relationship between serum level of oxidized LDL antibodies and CAD. Five out of five cross-sectional studies that studied the association between level of immunoglobulin (Ig) M anti-oxidized LDL and degree of CAD as measured in patients undergoing clinically indicated CAG, showed that IgM is inversely associated with imaged degree of CAD. In addition, IgM anti-oxidized LDL also appeared inversely associated with the incidence of long-term CV events in patients without objectified CAD, as found in three of the six studies that assessed this association. No associations were found in the two studies conducted in patients with objectified CAD. With regard to IgG anti-oxidized LDL, inconclusive results were described in the current literature and study methods varied greatly. We concluded that IgM anti-oxidized LDL may be of interest for identifying high-risk IHD patients.

Conclusions

Part I of this thesis has showed that, in patients with established IHD, in particular coronary atherosclerotic plaque burden carries prognostic value for the incidence of recurrent CV events. Noteworthy, we showed that the atherosclerotic burden of one single non-culprit coronary artery segment reflects the burden of the complete coronary tree, and that IVUS and NIRS imaging of this segment may be used for long-term prognostication in IHD patients. Our finding that plaque morphology (i.e. the presence of vulnerable plaques at baseline) was unrelated to the incidence of future CV events may be (partly) explained by the fact that coronary plaque vulnerability is a dynamic progress. Probably, plaque vulnerability aggravates or stabilizes in the presence or absence of widespread inflammation. Lastly, we found that SSII may be used for prognostication purposes in CAD patients irrespective of CAD complexity.

In part II of this dissertation, we have found several biomarkers, including ST2, GDF15 and the larger VLDL-P's, that were associated with recurrent CV events in IHD patients and we showed that repeated measurements of these markers may carry incremental information for prognostication. However, contrary to our expectations we have not identified biomarkers that show a sudden increase prior to recurrent CV events. Hence, so far, we have not found biomarkers that may be used to monitor IHD patients and identify high-risk episodes for CV events. Ultimately, enhancing a more precision-based, personalised approach to prognostication in patients with IHD and their clinical care still has many challenges. Although the concept of patient monitoring using biomarkers appears promising, it remains to be established if this concept may actually manifest in clinical practice.

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Nederlandse samenvatting

Onderwerp van dit proefschrift is de identificatie van patiënten met hart- en vaatziekten, in het bijzonder patiënten met een ischemische hartziekte, die een verhoogd risico hebben op herhaling van een klinisch voorval, zoals bijvoorbeeld een acuut coronair syndroom (ACS). Binnen het eerste jaar na een ziekenhuisopname vanwege een ischemische hartziekte, is de kans op overlijden ten gevolge van een terugkerende cardiovasculaire (CV) voorval 9%. Wij stelden dat een meer precieze, patiëntgebonden benadering van het ziekteverloop in deze patiënten het doen van een risicovoorspelling, en waar mogelijk uiteindelijk secundaire preventie, zou kunnen verbeteren.

Beeldvorming van coronaire atherosclerose bij patiënten met ischemische hartziekte

Aan de hand van invasieve beeldvormingsmodaliteiten zoals coronaire angiografie (CAG), intravasculaire echografie (IVUS) en nabij-infrarood spectroscopie (NIRS), hebben wij de verdeling van coronaire atherosclerose en de morfologie van coronaire laesies bestudeerd en hebben we hun relatie met een ongunstige klinische langetermijnprognose onderzocht bij patiënten na een ziekenhuisopname wegens ACS of stabiele angina pectoris (SAP).

De SYNTAX-score is een op CAG-gebaseerde risicoscore ter kwantificatie van de complexiteit van coronairlijden, waarin zowel luminale stenoses als andere factoren die de ernst van coronairlijden weerspiegelen, zoals laesielengte en –spreiding, wordt meegenomen. De SYNTAX-score is beperkt tot coronaire luminografie en kan daarom op zichzelf de morfologie van laesies niet kwalificeren. In **hoofdstuk twee** onderzochten we 680 ACS/SAP patiënten en vonden een verband tussen de complexiteit van het coronairlijden van een patiënt, zoals gekwantificeerd met de SYNTAX-score (op basis van alle drie de coronairarteriën) en met verschillende laesieparameters gemeten middels IVUS en NIRS, verkregen uit een enkel *non-culprit* coronairsegment. De SYNTAX-score correleerde goed, in het bijzonder met IVUS-gemeten *fibrous* en *fibro-fatty* weefsel en NIRS-gemeten *lipid burden*. We vonden geen associatie tussen de SYNTAX-score en de aanwezigheid van *thin-cap fibroatheroma* (TCFA). Een belangrijke secundaire bevinding was dat beeldvorming van een enkel coronairsegment afdoende zou kunnen zijn voor een adequate beoordeling van de ernst van het coronairlijden van een patiënt.

De SYNTAX-score kan gebruikt worden voor het voorspellen van klinische CV voorvallen tot vijf jaar na een percutane interventie (PCI) of bypass operatie (CABG). De prognostische waarde van IVUS- en NIRS-gemeten parameters was tot nu toe echter alleen bekend tot één jaar na ziekenhuisopname. Derhalve hebben we van zowel IVUS- als van NIRS-gemeten parameters, verkregen uit een *non-culprit* coronair segment, de lange termijn prognostische waarde onderzocht. Bij de 275 patiënten die

NIRS-beeldvorming ondergingen op *baseline*, zoals beschreven in **hoofdstuk drie**, was elke toename van 100 eenheden *lipid core burden index* (in een coronairsegment van 4 mm lengte) gerelateerd aan een 19% toename in incidentie van klinische CV voorvallen binnen vier jaar. Bij de 581 patiënten die IVUS-beeldvorming ondergingen, zoals beschreven in **hoofdstuk vier**, vonden we dat zij met een laesie van een minimaal lumen oppervlak ≤ 4.0 mm2 op *baseline*, een verhoogde kans op klinische CV voorvallen hadden van 49%. Daarbij was elke toename van 10 eenheden in *plaque burden* geassocieerd met een 26% toename in incidentie van klinische CV voorvallen binnen vierenhalf jaar. Zoals al gesuggereerd in **hoofdstuk twee,** bleken dus vooral de uitgebreidheid van een coronaire laesie als ook de *lipid burden* belangrijke voorspellende factoren voor een ongunstige prognose, en in mindere mate de morfologie van een laesie. Verder bevestigden we dat beeldvorming van een enkel coronairsegment afdoende zou kunnen zijn om uitspraken te doen over de prognose van een ACS/SAP patiënt.

Ten slotte hebben we in **hoofdstuk vijf** de prognostische waarde van de SYNTAX-score II (SSII) op de lange termijn bestudeerd. De SSII is een risicoscore die is opgebouwd uit zowel de anatomische SYNTAX-score als ook klinische variabelen waaronder leeftijd, geslacht, creatinineklaring, linkerventrikelejectiefractie, het hebben van perifeer vaatlijden, chronische obstructieve longziekte en hoofdstamlijden. De SSII was eerder al gevalideerd voor het voorspellen van mortaliteit in patiënten met drievatslijden. Echter, of de SSII ook kon worden toegepast in een meer heterogene populatie, zoals bij patiënten met eenvats- of tweevatslijden, was onbekend. Daarom onderzochten wij de prognostische waarde van de SS2 in 628 patiënten met één- of tweevatslijden die een PCI ondergingen vanwege ACS of SAP. In onze studie vonden we dat elke punt toename in SSII correspondeerde met een verhoogd mortaliteitsrisico van 10% binnen vierenhalf jaar. De *C-index* van de SSII was 0.77, wat kan worden geclassificeerd als goed. De SSII kan dus worden toegepast voor het voorspellen van het risico op overlijden binnen vijf jaar na een PCI, ongeacht de uitgebreidheid van het coronairlijden.

De rol van bloedbiomarkers bij patiënten met coronairlijden

Het meten van biomarkers in het bloed van patiënten met coronairlijden zou een non-invasieve manier kunnen zijn om het proces van coronairlijden per patiënt nauwkeurig te volgen over de tijd. Momenteel zijn er verschillende biomarkers geïdentificeerd die mogelijk geassocieerd zijn met de ernst van coronairlijden en met een verhoogd risico op het ontwikkelen van klinische CV voorvallen. De bestaande studies richten zich echter vooral op de voorspellende waarde van een eenmalige biomarkermeting, bijvoorbeeld gemeten tijdens een ziekenhuisopname op *baseline*. Bij deze studieopzet wordt er geen rekening gehouden met het dynamische karakter van coronairlijden en

blijft het onbekend hoe een biomarker zich gedraagt gedurende ziektebeloop. Wij stelden dat dergelijke informatie nodig is om te onderzoeken of een biomarker, eventueel in een *multi-marker setting*, gebruikt zou kunnen worden voor identificatie en monitoring van hoog-risico patiënten met coronairlijden. Daarom bestudeerden we in **hoofdstuk zes** de temporele patronen van *Myeloperoxidase* en *Galectin-3* in 187 patiënten die een ACS hebben doorgemaakt voorafgaand aan een tweede klinische CV gebeurtenis gedurende een jaar *follow-up*. *Myeloperoxidase* en *Galectin-3* zijn twee biomarkers die eerder zijn geassocieerd met de kwetsbaarheid van coronaire laesies. Met behulp van herhaalde bloedmetingen, hebben we de evolutie van beide biomarkers over de tijd, zowel vlak na een ACS als in aanloop naar een volgend ACS in kaart kunnen brengen. We vonden dat Myeloperoxidase een piek vertoonde van 78.3ng/ml op het moment van ziekenhuisopname en vervolgens afnam en stabiliseerde binnen zeven dagen na opname. Vervolgens bleef *Myeloperoxidase* stabiel op een waarde van 25.6 ng/ml (IQR: 20.4-32,0) gedurende de complete *follow-up*. Bovendien, werd er geen toename in *Myeloperoxidase* gezien voorafgaand aan een recidiverende klinische CV gebeurtenis. *Galectin-3* liet gedurende de complete *follow-up* een stabiele waarde zien van gemiddeld 0.23 (0.17-0.30) ng/ml. Ten slotte vonden we dat de longitudinale waardes van *Myeloperoxidase* en *Galectin-3* niet geassocieerd waren met recidiverende klinische CV gebeurtenissen.

In **hoofdstuk zeven** onderzochten we het temporele patroon van *Circulating Growth differentiation Factor-15* (GDF-15) bij 111 patiënten opgenomen wegens een ACS. GDF-15 is een inflammatoire biomarker die wordt uitgescheiden in het hart tijdens overbelasting van de hartspier en welke in eerdere studies van prognostische waarde bleek te zijn in patiënten na een ACS. GDF-15 was gedurende het jaar *follow-up* systematisch hoger bij patiënten die recidiverende CV gebeurtenissen ontwikkelden (eindpuntpatiënten), dan in degenen die dat niet deden. 30 Dagen na de *baseline* ACS ziekenhuisopname, hadden eindpuntpatiënten GDF-15 waardes die gemiddeld 333 (95% CI 68-647) pg/ml hoger waren dan in controlepatiënten (1704 versus 1371 pg/ml). Er was een grote variabiliteit in GDF-15waardes tussen de patiënten (82% in eindpuntpatiënten en 84% in controlepatiënten), daarentegen was de variabiliteit in GDF-15 waardes binnen patienten laag (18% in eindpuntpatiënten en 16% in controlepatiënten). Wij concludeerden dat GDF-15 gebruikt zou kunnen worden voor risicovoorspellingen bij patiënten die een ACS hebben doorgemaakt, waarbij het gebruik van individuele referentiewaardes de voorkeur zouden hebben.

Hoofdstuk acht beschreef het temporele patroon van soluble *Suppression of Tumorigenicity-2* (sST2) gedurende één jaar bij 187 patiënten na opname wegens een ACS. sST2 is een biomarker voor toenemende overbelasting van myocyten en eerder onderzoek heeft aangetoond dat het hebben van hogere sST2 waardes geassocieerd is met een slechte prognose in patiënten met ischemische hartziekte. Door middel van

herhaalde metingen vonden wij een verband tussen het langdurig hebben van hogere sST2 waardes na een ACS en recidiverende CV gebeurtenissen. Een toename van één standaardafwijking in sST2 waarde correleerde in onze studie met een risicotoename van 64% op het krijgen van een recidiverende klinische CV gebeurtenis. Verder zagen we dat de waardes per patiënt weinig verschilden over de tijd, waaruit we concludeerden dat een beperkt aantal metingen per patiënt voldoende zou kunnen zijn voor het voorspellen van een prognose.

Naast bovengenoemde biomarkers die al eerder geïdentificeerd waren als mogelijke markers voor CV voorvallen in patiënten met ischemische hartziekte, hebben we ook een breed scala aan onbekende eiwit biomarkers geanalyseerd in patiënten die opgenomen waren wegens een ACS. De immunoassay van Olink Proteomics werd gebruikt om waardes van deze onbekende eiwit biomarkers te verkrijgen, welke werden uitgedrukt in een arbitraire eenheid op de log2-schaal (*Normalised Protein eXpression* [NPX]). In **hoofdstuk negen** beschreven we bij 131 patiënten na ACS opname de temporele patronen van 25 eiwitten die de *mitogen-activated protein kinase* (MAPK) cascade stimuleren. De MAPK-cascade is een intracellulaire cascade die gemoduleerd kan worden door bloedbiomarkers om intracellulaire processen in gang te zetten, zoals celgroei, -differentiatie of -apoptose. Eerdere studies hebben aangetoond dat de MAPK-cascade een rol speelt bij vasculaire laesievorming, myocytpathologie door overbelasting en herstenose na stenting. Wij vonden dat de serumwaarde van *angiopoietin-1* (ANG-1), *proteinase-activated receptor 1* (PAR-1) en *bone morphogenetic protein 6* (BMP-6) hoger was in de patiënten die binnen 30 dagen na ziekenhuisopname wegens ACS een tweede CV voorval doormaakten. Na deze 30 dagen was geen van de 25 eiwitten significant verhoogd in de eindpuntpatiënten. In deze zelfde patiëntengroep van 131 patiënten beschreven we in **hoofdstuk tien** 29 immunologische en inflammatoire eiwitten en vonden dat de serumwaardes van *C-X-C motif chemokine 1* (CXCL1), *slam-family member 5* (CD84) en *tumor necrosis factor receptor superfamily member 10a* (TNFRSF10A) ook hoger waren in de patiënten die een recidiverende CV gebeurtenis doormaakten binnen 30 dagen na een ziekenhuisopname wegens ACS, maar dit was niet het geval na deze 30 dagen. Verder liet geen van de onderzochte eiwitten beschreven in **hoofdstuk negen of tien** een geleidelijke of plotselinge toename in serumwaarde zien voorafgaand aan een recidiverend klinisch CV voorval.

Hoofdstuk elf focuste wederom op het verkennen van nieuwe markers, echter ditmaal door het herhaald meten van (lipide) metabolieten in 158 patiënten na ziekenhuisopname wegens ACS. Voor deze analyses maakten we gebruik van geautomatiseerde proton NMR-spectroscopie om per serummonster tegelijkertijd 151 metabolieten te kwantificeren. Elke toename in standaardafwijking in de partikelconcentraties van *extremely large very low density lipoprotein* (VLDL), *very large* VLDL and *large* VLDL was respectievelijk gerelateerd aan een 60%, 60% en 56% verhoogde incidentie in

recidiverende CV gebeurtenissen binnen een jaar na ziekenhuisopname wegens ACS. Bovendien zagen we dat in de eindpuntpatiënten de partikelconcentraties van *extremely large* VLDL, *very large* VLDL en *large* VLDL geleidelijk toenamen voorafgaand aan een CV gebeurtenis. Het herhaaldelijk meten van deze VLDL partikelconcentraties in patiënten na een opname wegens ACS zou dus relevante informatie kunnen opleveren voor risicovoorspellingen in deze patiënten.

Het verlagen van LDL-cholesterol in patiënten met ischemische hartziekte vermindert het risico op recidiverende klinische CV voorvallen. Amerikaanse en Europese richtlijnen raden aan om LDL-waardes in hoogrisicopatiënten met hart- en vaatziekten onder de 1.8 mmol/L te houden. Tot nu toe hebben eerdere studies alleen de variabiliteit in LDL waardes bestudeerd in gezonde patiënten. De resultaten van onze analyse uitgevoerd bij 157 patiënten die na een ACS behandeld werden met statines, zoals beschreven in **hoofdstuk twaalf**, toonden een *reference change value* (RCV) voor LDL van 32.7%. Deze RCV impliceert dat een patiënt met een LDL waarde van 1.8 mmol/l tijdens klinische metingen kan variëren tussen 1.2 mmol/l en 2.4 mmol/l door natuurlijke en analytische variatie. Omdat deze variatie, indien onbekend, zou kunnen leiden tot het onjuist aanpassen van de cholesterolbehandeling van een patiënt, moet de intra-individuele variabiliteit in LDL in acht worden genomen bij het doorvoeren van veranderingen in de cholesterolbehandeling van een patiënt die behandeld wordt met statines.

Tot slot hebben we in **hoofdstuk dertien** systematisch de huidige beschikbare literatuur in kaart gebracht wat betreft de relatie tussen serumwaardes van geoxideerde LDL-antilichamen en coronairlijden. Vijf van de vijf cross-sectionele studies die de associatie tussen het niveau van immunoglobuline (Ig) M-anti-geoxideerd LDL en de mate van coronairlijden onderzochten in patiënten die een klinische CAG ondergingen, vonden dat IgM omgekeerd evenredig geassocieerd was met de ernst van coronairlijden zoals gekwantificeerd tijdens CAG. Hiernaast leek IgM-anti-geoxideerd LDL ook omgekeerd evenredig geassocieerd te zijn met de incidentie van klinische CV gebeurtenissen in patiënten zonder CV voorgeschiedenis, zoals beschreven werd in drie van de zes studies die deze associatie onderzochten. In de twee studies die deze associatie bestudeerden in patiënten met reeds bestaande ischemische hartziekte werden geen associaties gevonden. Wat betreft IgG-anti-geoxideerd LDL zijn er tot dusver tegenstrijdige bevindingen beschreven in de literatuur waarbij er ook een sterke variatie werd gezien in de gebruikte studiemethoden. We concludeerden dan ook dat alleen IgM-anti-geoxideerd LDL geschikt zou kunnen zijn voor het identificeren van patiënten met een verhoogd risico op een toekomstig CV gebeurtenis.

Conclusies

Deel I van dit proefschrift toonde aan dat in patiënten met ischemische hartziekte voornamelijk de coronaire *plaque burden* belangrijk is voor het voorspellen van toekomstige klinische CV gebeurtenissen. Daarnaast hebben we aangetoond dat de *plaque burden* van een enkel *non-culprit* coronairsegment afdoende zou kunnen zijn om de ernst van het totale coronairlijden van een patiënt in te schatten. IVUS- en NIRS-beeldvorming van dit segment kan gebruikt worden voor het doen van lange termijn risicovoorspellingen in patiënten met ischemische hartziekte. Onze bevinding dat laesiemorfologie (dat wil zeggen de aanwezigheid van kwetsbare laesies tijdens een ACS) niet gerelateerd is aan de incidentie van toekomstige klinische CV gebeurtenissen kan (gedeeltelijk) worden verklaard door het feit dat kwetsbare laesies dynamisch van aard zijn en kunnen stabiliseren over de tijd. Waarschijnlijk neemt de kwetsbaarheid van laesies toe of stabiliseren deze juist in de aan- of afwezigheid van algehele (cardiale) inflammatie. Ten slotte hebben we geconstateerd dat SSII kan worden gebruikt voor prognosedoeleinden in patiënten met coronairlijden, ongeacht een- twee- of drievatslijden.

In deel II van dit proefschrift hebben we verschillende biomarkers onderzocht, waaronder ST2, GDF15 en de grotere VLDL partikels, die geassocieerd zijn met recidiverende klinische CV voorvallen in patiënten na een ACS opname. We toonden aan dat het herhaald meten van deze biomarkers mogelijk van toegevoegde waarde kan zijn voor het doen van risicovoorspellingen. In tegenstelling tot onze verwachting, hebben we geen (nieuwe) biomarkers gevonden die een plotselinge stijging lieten zien voorafgaand aan een recidiverende CV gebeurtenis. Met ons huidige onderzoek hebben we nog geen biomarkers kunnen identificeren die geschikt zouden kunnen zijn voor het monitoren van patiënten na een ACS opname en die gebruikt zouden kunnen worden voor het herkennen van risicovollere periodes. Uiteindelijk kent het ontwikkelen van een meer precieze, patiëntgebonden prognose als ook de behandeling van deze patiëntengroep door middel van biomarkers nog steeds vele uitdagingen. Hoewel het concept van biomarkergeleide monitoring van patiënten veelbelovend klinkt, zal nog moeten blijken of dit concept zich daadwerkelijk kan realiseren in de klinische praktijk.

List of publications

1. **Vroegindewey MM**, Schuurman AS, Oemrawsingh RM, van Geuns RJ, Kardys I, Ligthart J, Daemen J, Boersma E, Serruys PW, Akkerhuis KM. SYNTAX score II predicts long-term mortality in patients with one- or two-vessel disease. PLoS One. 2018;13(7):e0200076.
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5. **Vroegindewey MM**, Schuurman AS, Kardys I, Anroedh SS, Oemrawsingh RM, Ligthart J, Garcia-Garcia HM, van Geuns RM, Regar E, Van Mieghem NM, Serruys PW, Boersma E, Akkerhuis M. SYNTAX score in relation to intravascular ultrasound and near-infrared spectroscopy for the assessment of atherosclerotic burden in patients with coronary artery disease. EuroIntervention. 2019;14(13):1408-15.
6. **Vroegindewey MM**, Buljubasic N, Oemrawsingh RM, Kardys I, Asselbergs FW, Van der Harst P, Umans VA, Kietselaer B, Lenderink T, Liem A, Mouthaan H, Boersma E, Akkerhuis KM. MAPK-cascade stimulating biomarkers in relation to recurrent coronary events following an acute coronary syndrome. J Mol Biomark Diagn. 2019.
7. **Vroegindewey MM**, van den Berg VJ, Bouwens E, Akkerhuis KM, Oemrawsingh RM, Asselbergs FW, Lenderink T, van der Harst P, Ronner E, Umans V, Kardys I, Boersma E. Temporal evolution of myeloperoxidase and galectin 3 during 1 year after acute coronary syndrome admission. Am Heart J. 2019.
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PhD portfolio

	Year	Workload (ECTS)
Master of Science in Epidemiology		
NIHES Research Master in Clinical Research	2015-2017	120.0
General PhD courses		
BROK course	2018	1.5
Scientific integrity	2018	0.3
In-depth cardiovascular courses		
COEUR Cardiovascular imaging and diagnostics	2016	1.5
COEUR Heart failure research	2016	1.5
COEUR Omics in cardiovascular medicine	2017	0.3
COEUR Aneurysmal diseases	2018	0.5
COEUR PhD day	2018	0.3
Conferences		
Optics in Cardiology, Rotterdam, The Netherlands	2017	0.6
ESC congress 2017, Barcelona, Spain	2017	1.5
NHI Translational Cardiovascular Research Meeting, Utrecht, The Netherlands	2018	0.6
ESC congress 2018, Munich, Germany	2018	1.5
Teaching activities		
Erasmus Anatomy Research Project (EARP), *Thoracic anatomy*	2016	3.0
Lecturing 1st year medical students of Erasmus MC, C*ardiovascular anatomy and imaging*	2016	1.0
Lecturing 2nd year nursing students, *Thoracic anatomy*	2016	0.6
Lecturing 1st and 2nd year students of Erasmus University College, *Thoracic anatomy*	2016-2018	0.5
Supervising 2nd year medical students of Erasmus MC, *Writing a systematic review*	2017-2018	0.6
Presentations		
COEUR seminar, *Enhancing precision medicine through protein biomarker profiling*	2017	0.3
ESC congress 2017, Barcelona, Spain	2017	0.3
ESC congress 2018, Munich, Germany	2018	0.6

About the author

Maxime Maria Vroegindewey was born on the 26th of January, 1993 in Rotterdam, the Netherlands. After graduating secondary school, she spent a year studying Biology at the University of Leiden and obtained her propedeuse. Thereafter, in 2012, she started medical school at the Erasmus University Rotterdam. After obtaining her bachelor's degree, she enrolled in the research master Clinical Research at the Netherlands Institute for Health Sciences (NIHES), Rotterdam. During this Msc program she worked on several research projects at the department of clinical epidemiology of cardiovascular diseases. In addition, she studied at the Graduate Summer Institute of Epidemiology and Biostatistics at the Johns Hopkins Bloomberg School of Public Health, Baltimore. After finishing the NIHES MSc program, she continued her research as a research fellow under supervision of prof. dr. ir. Eric Boersma on imaging and biomarkers of coronary atherosclerosis in patients with ischemic heart disease.
Maxime will graduate medical school in the summer of 2021.

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