

BMJ Open Cohort profile of BIOMArCS: the BIOMarker study to identify the Acute risk of a Coronary Syndrome – a prospective multicentre biomarker study conducted in the Netherlands

Rohit M Oemrawsingh,^{1,2,3} K Martijn Akkerhuis,^{1,2} Victor A Umans,⁴ Bas Kietzelaer,⁵ Carl Schotborgh,⁶ Eelko Ronner,⁷ Timo Lenderink,⁸ Anho Liem,⁹ David Haitzma,¹⁰ Pim van der Harst,¹¹ Folkert W Asselbergs,^{12,13,14} Arthur Maas,¹⁵ Anton J Oude Ophuis,^{16,17} Ben Ilmer,¹⁸ Rene Dijkgraaf,¹⁹ Robbert-Jan de Winter,²⁰ S Hong Kie The,²¹ Alexander J Wardeh,²² Walter Hermans,²³ Etienne Cramer,²⁴ Ron H van Schaik,¹ Imo E Hoefler,¹² Pieter A Doevendans,³ Maarten L Simoons,¹ Eric Boersma^{1,2}

To cite: Oemrawsingh RM, Akkerhuis KM, Umans VA, *et al.* Cohort profile of BIOMArCS: the BIOMarker study to identify the Acute risk of a Coronary Syndrome – a prospective multicentre biomarker study conducted in the Netherlands. *BMJ Open* 2016;**6**:e012929. doi:10.1136/bmjopen-2016-012929

► Prepublication history for this paper is available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2016-012929>).

Received 2 June 2016
Revised 15 September 2016
Accepted 13 October 2016



CrossMark

For numbered affiliations see end of article.

Correspondence to
Professor Eric Boersma;
h.boersma@erasmusmc.nl

ABSTRACT

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

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

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

Strengths and limitations of this study

- The only currently available study cohort of patients with coronary artery disease with such high frequency blood sampling in order to investigate multiple biomarker patterns *shortly prior* to a coronary ischaemic event.
- Prospective sample collection with ‘retrospective’ biomarker and genetic analyses after event adjudication.
- Strict and prespecified study/laboratory processing protocol minimising preanalytical confounding.
- The BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS) does not aim to unravel whether certain biomarkers are merely markers reflecting pathways of disease, or mediators that are directly involved within distinct pathophysiological cascades in the arterial wall.

Future plans and dissemination: Follow-up and event adjudication have been completed. Prespecified biomarker analyses are currently being performed and dissemination through peer-reviewed publications and conference presentations is expected from the third quarter of 2016. Should identification of a ‘vulnerable period’ prove to be feasible, then future research could focus on event reduction through pharmacological or mechanical intervention during such periods of high risk for ACS.

Trial registration number: NTR1698 and NTR1106.

INTRODUCTION

Generalised cardiovascular (CV) risk assessment models have proven to be valuable for

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

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

Therefore, we hypothesised that divergent biomarker patterns, detected through ambulatory and highly frequent blood sampling, could identify patients in a 'vulnerable period' for the occurrence of an imminent myocardial infarction (MI). In order to investigate this hypothesis, our aim was to obtain serial biomarker measurements as closely as possible prior to an ischaemic event, yet in a phase in which the patient is still asymptomatic. Subsequent analysis of serial biomarker patterns up to the coronary event should elucidate biomarker kinetics, patterns, appropriate cut-off values and prediction characteristics (such as time frames), particularly shortly *prior* to the actual occurrence of an ACS.

COHORT DESCRIPTION

Study objectives

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

Study cohort

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

Blood samples were collected at admission, at the day of hospital discharge and subsequently every fortnight during the first 6 months after discharge, followed by monthly blood sample collection until 1 year. Patients were offered some flexibility in the follow-up scheme: visit windows are ± 1 week, and a maximum of two consecutive visits are allowed to be skipped (for personal reasons). If logistic circumstances hindered inclusion during hospitalisation, patients could be included on the first outpatient visit within 6 weeks after discharge. The sample collection schedule was then adapted accordingly. Follow-up blood sampling was terminated permanently after coronary artery bypass grafting (CABG), hospital admission for heart failure or a deterioration of renal function leading to a glomerular filtration rate <30 mL/min/1.73 m², in order to minimise bias in circulating biomarker concentrations. During the course of the study, we observed prespecified discontinuation of biomarker sampling in 13 patients who were revascularised through CABG at a median follow-up duration of 116 days after the index ACS. In these patients, samples were taken up until the bypass operation.

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

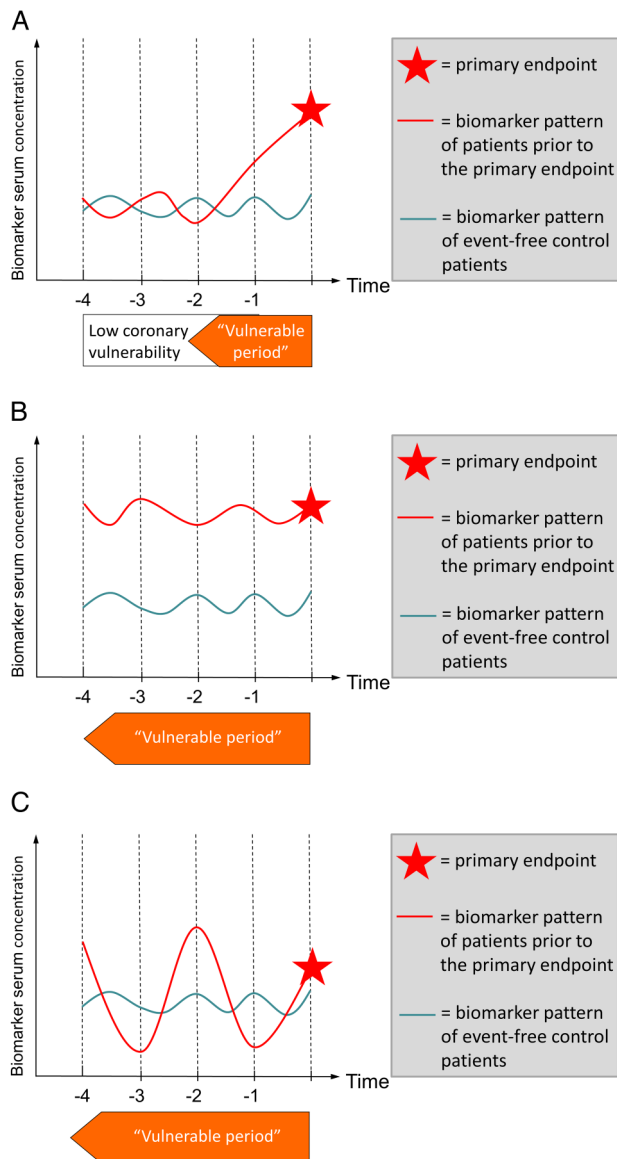


Figure 1 Different hypothetical scenarios of biomarker evolution during stable and vulnerable periods in the lifetime of a patient with coronary artery disease. Panel A describes a scenario in which biomarker patterns are relatively stable in a period of low coronary vulnerability, but are clearly divergent and upregulated shortly prior to the primary end point. Panel B describes a potential scenario in which the 'vulnerable period' for a coronary event is relatively longer and characterised by persistently higher biomarker levels. Depending on the specific biomarker, this scenario could also apply in case of persistently lower (instead of higher) levels. Panel C depicts a divergent biomarker pattern in which a high degree of variability is associated with an increased risk of adverse cardiac outcome. Naturally, numerous variations and combinations of the aforementioned scenarios can be proposed for each specific biomarker depending on its characteristic pattern and kinetics.

This is an observational study. As such, it does not interfere with patient treatment. All patients were treated as per prevailing guidelines and at the discretion of the investigator. Patients were only included after they

provided written informed consent. The consent enables the investigators to enquire on the patients' health status up to 15 years after enrolment.

Blood sample collection

Blood samples were first handled and securely stored on-site. After preparation, aliquots were frozen at -80°C within 2 hours after withdrawal. Long-term storage and biomarker analysis will take place at the department of Clinical Chemistry of the Erasmus MC. Apart from storage of serum, citrate-plasma and EDTA-plasma, the BIOMArCS laboratory protocol also foresaw in collection and preservation of leucocytes for the purpose of genome analyses and flow cytometric measurements of certain circulating leucocyte (monocyte) subsets that are thought to reflect endothelial regenerative capacity.²¹

Study end points

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

Sample size considerations

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

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

Table 1 Inclusion and exclusion criteria**Inclusion: a patient must meet all criteria**

- 1 Age ≥ 40 years
- 2 Symptoms of typical ischaemic chest pain, lasting 10 min or more within the preceding 24 hours prior to presentation
- 3a ECG: (non-)persistent ST segment elevation >1.0 mm in two or more contiguous leads, or dynamic ST segment depression >1.0 mm in two or more contiguous leads, *OR*
- 3b Biochemical evidence of myocardial injury: CKMB or (high-sensitivity) troponin I or (high-sensitivity) troponin T elevation according to the applicable ESC guidelines of non ST-elevation acute coronary syndromes
- 4 Presence of at least one of the following risk factors: age ≥ 75 years, diabetes, prior cardiovascular disease, prior cerebrovascular disease and prior peripheral arterial disease. In addition, other risk factors mentioned below can be considered as well, but each only counts as half a risk factor, that is, two of these are required for inclusion: age ≥ 65 years in men, age ≥ 70 years in women, hypertension, hypercholesterolaemia, current smoking or microalbuminuria,* positive family history of coronary artery disease.†
- 5 Written informed consent

Exclusion: a patient cannot be included in case of any of the criteria below

- 1 Myocardial ischaemia precipitated by a condition other than atherosclerotic coronary artery disease
- 2 Left ventricular ejection fraction $<30\%$, or end-stage congestive heart failure (NYHA class III or IV)
- 3 Renal dialysis, or severe chronic kidney disease with measured or calculated GFR (Cockcroft-Gault or MDRD4 formula) of <30 mL/min/1.73 m²
- 4 Coexistent condition with life expectancy <1 year or otherwise not expected to complete follow-up

*Defined as >2.5 to 25 mg albumin/mmol creatinine for men and >3.5 to 35 mg for women, or >20 to 200 mg/L urinary albumin concentration in a single urine sample.

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

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

Table 2 Baseline characteristics of the entire cohort of 844 patients*Presentation and initial treatment*

Age, years	62.5 (54.3, 70.2)
Man	77.9
Admission diagnosis	
STEMI	51.7
NSTEMI	37.7
UAP	10.6
Culprit artery	
RCA	33.1
LM	2.5
LAD	31.9
LCX	16.5
Coronary angiography performed	94.4
Percutaneous coronary intervention	86.3
Maximum CK during admission (iU/L)	513 (200, 1370)

Cardiovascular risk factors

Current smoking	40.5
Diabetes mellitus	23.5
Hypertension	55.5
Hypercholesterolaemia	49.3

Cardiovascular history

Prior percutaneous coronary intervention	26.2
Prior coronary artery bypass grafting	10.0
Prior myocardial infarction	26.9
Prior heart failure	2.4
Valvular heart disease	2.2
Prior stroke	9.0
Peripheral artery disease	8.9

Medication at first blood sample moment

Aspirin	95.3
P2Y12 inhibitor	95.2
Vitamin K antagonist	6.8
Statin	96.2
β -blocker	89.8
ACE inhibitor or ARB	82.9

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

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

slope of 0.06–0.11 mmol/L/month can be demonstrated between cases and non-cases with a power of 80% (two-sided test with an α error of 5%). We judged that these differences are small in clinical terms, and we considered the observed variations in LDL-C levels representative of changes in other biomarkers. In order to obtain 50 cases, given the anticipated incidence, a total of 700–1000 patients needed to be enrolled.

Construct of the case-cohort analysis set

A random, representative sample of 150 patients (random subcohort) will be chosen from all enrolled patients, and the patients who reach a study end point

will be added. We anticipate that $(50/1000) \times 150 = 8$ to $(50/700) \times 150 = 11$ patients of the random subcohort will reach the primary end point. Hence, the expected ratio between patients with and without the primary study end point in the analysis set will be 1:2.8–1:2.9, which allows us to reveal clinically relevant differences in biomarker patterns with sufficient statistical power (see *Sample size considerations* section above).

Biomarker selection and significance testing

Atherosclerosis and plaque destabilisation leading to intracoronary thrombosis and an ACS is the result of a very heterogeneous process with many intertwined

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

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

constituents. Vascular inflammation and endothelial disruption can result in thrombosis, which in turn can exacerbate inflammation.¹¹ Many of the circulating biomarkers that have been shown to adequately predict risks of future CV events are therefore thought to reflect one or more of these distinct yet interdependent pathophysiological processes more or less specifically. Currently, markers like those mentioned in **box 1** are considered to have high potential, and will be determined and reported in prespecified consecutive phases. Their selection is hypothesis-driven and based on current literature, which is mainly based on one *single* measurement in time.^{13–19 23–30} The development of biomarker levels shortly after presentation for ACS, and, more importantly, the frequently sampled biomarker patterns during the (asymptomatic) period preceding a subsequent event are unknown. A call for epidemiological research to establish the clinical value of serial analysis of biomarkers in atherosclerotic disease during long-term follow-up has repeatedly sounded,^{12 31 32} but has not been answered as yet.

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

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

Aetiological and prognostic analyses of selected biomarkers

Compared with an analysis of the entire cohort, the advantage of a case-cohort design lies in its efficiency, whereas the ability to calculate absolute risks and rates is maintained.²² We will perform aetiological as well as prognostic analyses. We will use the framework of linear mixed-effects

models to assess changes in biomarker levels over time, while accounting for the correlation between repeated follow-up measurements in each patient.³³ For both the fixed-effects and random-effects parts of the model, we will test for possible non-linear evolutions, which will be modelled by restricted cubic splines.

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

Both univariate and multivariate analyses will be applied. The biomarker trajectories in the linear models will be adjusted (1) for age and sex, (2) GRACE risk score, (3) kidney function, (4) body mass index, diabetes mellitus, prior CAD, prior cerebrovascular disease and prior peripheral vascular disease, and (5) other variables that appear related to biomarker levels in the analysis set, to the extent that is permitted given the number of observations. The relation between biomarkers and study end points in the Cox model will be adjusted for GRACE risk score and prognostic biomarkers to the extent that is permitted, given the number of end point cases. For the purpose of multivariate adjustment, we will select the specific GRACE risk model that is best in line with the purpose of our study, namely an assessment of postdischarge death and MI. That particular GRACE risk model consists of age, troponin (or CKMB) elevation at admission, history of MI, congestive heart failure and whether CABG was performed at the index hospitalisation.³⁶

Risk models

Based on the results of the analyses above, multibiomarker models will be constructed to predict the risk of the study end points based on the temporal evolution of

Box 1 Biomarker selection

The following biomarkers are considered of high potential with regard to the BIOMArCS study to identify the Acute risk of a Coronary Syndrome (BIOMArCS) hypothesis and will be determined and reported in prespecified consecutive phases. Their selection is hypothesis-driven and based on current literature.

Phase 1

High sensitivity C reactive protein¹
 High sensitivity troponin I¹
 High sensitivity troponin T²
 NT-pro BNP³
 ST-2⁴
 Creatinine¹
 Total cholesterol, high-density lipoprotein cholesterol¹,
 low-density lipoprotein cholesterol⁵

Phase 2 (in alphabetical order)

Copeptin
 Ceramide (d18:1/16:0) as well as the following ceramide ratios:
 Cer(d18:1/16:0)/Cer(d18:1/24:0)
 Cer(d18:1/20:0)/Cer(d18:1/24:0)
 Cer(d18:1/24:1)/Cer(d18:1/24:0)
 Cystatin-C
 Galectin-3
 Growth differentiation factor-15
 Interleukins 1, 6, 8, 10, 18
 Monokine-induced by interferon- γ
 Myeloperoxidase
 Placental growth factor
 Plasminogen activator inhibitor 1
 Pregnancy-associated plasma protein A
 Regulated on activation normal T cell expressed and secreted
 Soluble CD40 ligand
 Tumour necrosis factor
 Von Willebrand factor

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

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

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

the biomarkers. We realise that the number of biomarkers (and covariates) will be limited by the number of end point events.³⁷

Early washout biomarker patterns and ancillary analyses

In an ancillary study of 68 patients (10% of the initially planned total study population of at least 700 patients), we aim to study the evolution/normalisation of biomarkers during the first 8 weeks after the index event. In these patients, (additional) blood samples are collected within 24, 48, 72 and 96 hours after admission, at the day of hospital discharge, and at 2, 4 and 8 weeks after discharge. Insight into these patterns will allow us to differentiate whether observed divergent biomarker

patterns prior to a repeat ACS during longer term follow-up are (partly) influenced by biochemical consequences of the index event.

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

Study organisation

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

Current status

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

DISCUSSION

Vulnerable period versus vulnerable plaque

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

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

Rationale behind the time intervals for sample collection

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

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

haemorrhage with deposition of erythrocyte-derived free cholesterol.¹⁰

Future directions

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

At a later stage (and dependent on the results of the aforementioned projects), the way could be paved towards intervention studies that evaluate the effectiveness and safety of a brief period of intensified medical treatment (or a percutaneous intervention) in order to prevent an otherwise imminent coronary event, as characterised by an abnormal 'high-risk' biomarker pattern. Future hypotheses could focus on plaque stabilisation or regression and endothelial repair in patients with 'high-risk' biomarker patterns such as a brief period of intravenous administration of apolipoprotein-A1 Milano,⁴⁶ proprotein convertase subtilisin/kexin type 9 inhibition,⁴⁷ or the use of the anti-inflammatory properties of P-selectin antagonists,⁴⁸ low-dose colchicine⁴⁹, low-dose methotrexate or interleukin-1 β inhibition.⁵⁰ Perhaps divergent biomarker patterns could be evaluated for selection of patients who benefit from prolonged dual antiplatelet therapy. Exogenous drugs such as agonists of vascular endothelial growth factor, peroxisomal proliferative-activated receptor agonists and granulocyte colony-stimulating factor, which exert their actions partly through endothelial progenitor cell-mediated re-endothelialisation may be of interest as well.⁵¹

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

Strengths and limitations

BIOMArCS is the only currently available study in which such frequent blood sampling has been performed on a

large scale in order to thoroughly investigate multiple biomarker patterns in patients with CAD. As such, BIOMArCS is conceptionally different from all other biomarker studies in patients with CAD, as it aimed to obtain blood samples as shortly as possible *prior* to a future adverse cardiac event. Although sample collection was performed prospectively, biomarker and genetic analyses will be performed retrospectively. As a dedicated biomarker study it benefits from a strict and prespecified laboratory processing protocol in which preanalytical confounding was minimised through standardisation of methods and materials for blood collection in all centres. Time from collection to standardised processing and freeze and thaw cycles for biomarker analyses are limited by protocol. Patients were interviewed at each venepuncture to inquire about their cardiac status and medication use, as well as about confounders of specific biomarkers (eg, new onset of other illnesses, infection, allergic reactions.)

It is important to emphasise that a clinical observational study such as BIOMArCS does not aim to unravel whether certain biomarkers are merely markers reflecting pathways of disease, or mediators that are directly involved within distinct pathophysiological cascades in the arterial wall. Definite delineation of biochemical events responsible for observed alterations in biomarker patterns prior to the end point, or final conclusions on mechanisms of disease are beyond the scope of this study design. In addition, our study was performed in patients with a known CAD. It is uncertain whether its conclusions may be extrapolated to the primary prevention setting.

Author affiliations

- ¹Erasmus MC, Rotterdam, The Netherlands
- ²Cardiovascular Research Institute COEUR, Rotterdam, The Netherlands
- ³Netherlands Heart Institute/Interuniversity Cardiology Institute Netherlands, Utrecht, The Netherlands
- ⁴Medical Center Alkmaar, Alkmaar, The Netherlands
- ⁵Maastricht University Medical Center, Maastricht, The Netherlands
- ⁶HagaZiekenhuis, The Hague, The Netherlands
- ⁷Reinier de Graaf Hospital, Delft, The Netherlands
- ⁸Zuyderland Hospital, Heerlen, The Netherlands
- ⁹Sint Franciscus Gasthuis, Rotterdam, The Netherlands
- ¹⁰Admiraal de Ruyter Hospital, Goes, The Netherlands
- ¹¹University of Groningen, University Medical Center Groningen, The Netherlands
- ¹²Division Heart & Lungs, Department of Cardiology, UMC Utrecht, Utrecht, The Netherlands
- ¹³Durrer Center for Cardiovascular Research, Netherlands Heart Institute, Utrecht, The Netherlands
- ¹⁴Faculty of Population Health Sciences, Institute of Cardiovascular Science, University College London, London, UK
- ¹⁵Gelre Hospital, Zutphen, The Netherlands
- ¹⁶Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
- ¹⁷Working Group on Cardiovascular Research the Netherlands (WCN), Utrecht, The Netherlands
- ¹⁸Havenziekenhuis, Rotterdam, The Netherlands
- ¹⁹St. Jansdal Hospital, Harderwijk, The Netherlands
- ²⁰Academic Medical Center, Amsterdam, The Netherlands
- ²¹Treant Zorggroep, locatie Bethesda, Hoogeveen, The Netherlands
- ²²Medisch Centrum Haaglanden locatie Westeinde, Den Haag, The Netherlands

²³St. Elisabeth Hospital, Tilburg, The Netherlands

²⁴Radboud University Medical Center, Nijmegen, The Netherlands

Contributors RMO, KMA, EB and MLS were responsible for the design of the BIOMArCS study. RMO drafted the manuscript. All other authors, KMA, EB, MLS, VAU, BK, CS, ER, TL, AL, DH, PvdH, FWA, AM, AJOO, BI, RD, R-JdW, SHKT, AJW, WH, EC, RHvS, IEH, PAD, revised it critically for important intellectual content and approved this version to be published. All authors contributed substantially to data acquisition for the BIOMArCS study and agree to be accountable for all aspects of this work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding The study is supported and funded by the Netherlands Heart Foundation (grant number 2007B012), the Netherlands Heart Institute-Interuniversity Cardiology Institute of the Netherlands (project number 071.01) and the Working Group on Cardiovascular Research Netherlands, all of which are non-commercial funding bodies. An unrestricted research grant is further obtained from Eli Lilly, the Netherlands. FWA is supported by a Dekker scholarship-Junior Staff Member 2014T001—the Netherlands Heart Foundation and UCL Hospitals NIHR Biomedical Research Centre.

Competing interests BIOMArCS was designed and initiated by the principal investigators. The trial will be conducted, and its results interpreted and reported independently of the aforementioned sponsors.

Ethics approval IRB of the ErasmusMC for national approval and subsequently the IRB of every participating institution for local approval.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Anyone can submit a prespecified analytical plan for biomarker analyses within the BIOMArCS data set to the principal investigator/Clinical Epidemiology Unit of the Erasmus MC Department of Cardiology. Biomarker analyses can only be performed after evaluation and written approval thereof by the BIOMArCS Executive Committee.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

1. Levy D, Wilson PW, Anderson KM, *et al.* Stratifying the patient at risk from coronary disease: new insights from the Framingham Heart Study. *Am Heart J* 1990;119:712–17; discussion 717.
2. Conroy RM, Pyörälä K, Fitzgerald AP, *et al.* Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;24:987–1003.
3. Boersma E, Pieper KS, Steyerberg EW, *et al.* Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation. Results from an international trial of 9461 patients. The PURSUIT Investigators. *Circulation* 2000;101:2557–67.
4. Antman EM, Cohen M, Bernink PJ, *et al.* The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. *JAMA* 2000;284:835–42.
5. Fox KAA, Fitzgerald G, Puymirat E, *et al.* Should patients with acute coronary disease be stratified for management according to their risk? Derivation, external validation and outcomes using the updated GRACE risk score. *BMJ Open* 2014;4:e004425.
6. Khot UN, Khot MB, Bajzer CT, *et al.* Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA* 2003;290:898–904.
7. Johnson KM, Dowe DA, Brink JA. Traditional clinical risk assessment tools do not accurately predict coronary atherosclerotic plaque burden: a CT angiography study. *AJR Am J Roentgenol* 2009;192:235–43.
8. Naghavi M, Libby P, Falk E, *et al.* From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part II. *Circulation* 2003;108:1772–8.

9. Davies MJ. Coronary disease: the pathophysiology of acute coronary syndromes. *Heart* 2000;83:361–6.
10. Ahmadi A, Leipsic J, Blankstein R, et al. Do plaques rapidly progress prior to myocardial infarction? The interplay between plaque vulnerability and progression. *Circ Res* 2015;117:99–104.
11. Libby P. Atherosclerosis: disease biology affecting the coronary vasculature. *Am J Cardiol* 2006;98:3Q–9Q.
12. Oemrawsingh RM, Akkerhuis KM, Boersma E. Tailor-made therapy for the prevention of acute coronary syndromes: future role of biomarkers in risk stratification and disease management. *Expert Rev Cardiovasc Ther* 2008;6:435–7.
13. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, et al. Relation of C-reactive protein to coronary plaque characteristics on grayscale, radiofrequency intravascular ultrasound, and cardiovascular outcome in patients with acute coronary syndrome or stable angina pectoris (from the ATHEROREMO-IVUS study). *Am J Cardiol* 2014;114:1497–503.
14. Oemrawsingh RM, Cheng JM, Garcia-Garcia HM, et al. High-sensitivity troponin T in relation to coronary plaque characteristics in patients with stable coronary artery disease; results of the ATHEROREMO-IVUS study. *Atherosclerosis* 2016;247:135–41.
15. Cheng JM, Oemrawsingh RM, Akkerhuis KM, et al. Circulating chemokines in relation to coronary plaque characteristics on radiofrequency intravascular ultrasound and cardiovascular outcome. *Biomarkers* 2014;19:611–19.
16. Battes LC, Akkerhuis KM, Cheng JM, et al. Circulating acute phase proteins in relation to extent and composition of coronary atherosclerosis and cardiovascular outcome: results from the ATHEROREMO-IVUS study. *Int J Cardiol* 2014;177:847–53.
17. Cheng JM, Suoniemi M, Kardys I, et al. Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: results of the ATHEROREMO-IVUS study. *Atherosclerosis* 2015;243:560–6.
18. Battes LC, Cheng JM, Oemrawsingh RM, et al. Circulating cytokines in relation to the extent and composition of coronary atherosclerosis: results from the ATHEROREMO-IVUS study. *Atherosclerosis* 2014;236:18–24.
19. Sonneveld MAH, Cheng JM, Oemrawsingh RM, et al. Von Willebrand factor in relation to coronary plaque characteristics and cardiovascular outcome. Results of the ATHEROREMO-IVUS study. *Thromb Haemost* 2015;113:577–84.
20. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, et al. PCSK9 in relation to coronary plaque inflammation: results of the ATHEROREMO-IVUS study. *Atherosclerosis* 2016;248:117–22.
21. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;353:999–1007.
22. Grobbee DE, Hoes AW. *Clinical epidemiology: principles, methods, and applications for clinical research*. Boston: Jones and Bartlett Publishers, 2009:259–61.
23. Kardys I, Oemrawsingh RM, Kay IP, et al. Lipoprotein(a), interleukin-10, C-reactive protein, and 8-year outcome after percutaneous coronary intervention. *Clin Cardiol* 2012;35:482–9.
24. Oemrawsingh RM, Lenderink T, Akkerhuis KM, et al. Multimarker risk model containing troponin-T, interleukin 10, myeloperoxidase and placental growth factor predicts long-term cardiovascular risk after non-ST-segment elevation acute coronary syndrome. *Heart* 2011;97:1061–6.
25. Everett BM, Brooks MM, Vlachos HEA, et al. Troponin and cardiac events in stable ischemic heart disease and diabetes. *N Engl J Med* 2015;373:610–20.
26. Giannitsis E, Spanuth E, Horsch A, et al. High-sensitivity cardiac troponin T and N-terminal pro-B-type natriuretic peptide predict mortality in stable coronary artery disease: results from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clin Chem Lab Med* 2013;51:2019–28.
27. Woitas RP, Kleber ME, Meintzer A, et al. Cystatin C is independently associated with total and cardiovascular mortality in individuals undergoing coronary angiography. The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Atherosclerosis* 2013;229:541–8.
28. Maiolino G, Rossitto G, Pedon L, et al. Galectin-3 predicts long-term cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2015;35:725–32.
29. Dieplinger B, Egger M, Haltmayer M, et al. Increased soluble ST2 predicts long-term mortality in patients with stable coronary artery disease: results from the Ludwigshafen risk and cardiovascular health study. *Clin Chemistry* 2014;60:530–40.
30. Hofer IE, Steffens S, Ala-Korpela M, et al. Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J* 2015;36:2635–42.
31. Sans S. In search of the grail: the never-ending story of biomarkers for coronary risk prediction. *Eur Heart J* 2004;25:1271–3.
32. MacFadyen RJ, Ng Kam Chuen MJ. Developing the utility of blood biomarker associations beyond population sample linkage to events in cardiovascular patients. *Eur Heart J* 2008;29:837–9.
33. Verbeke G, Molenberghs G. *Linear mixed models for longitudinal data*. New York: Springer-Verlag, 2000.
34. Rizopoulos R. *Joint models for longitudinal and time-to-event data with applications in R*. London: Chapman and Hall/CRC, 2012.
35. Ganna A, Reilly M, de Faire U, et al. Risk prediction measures for case-cohort and nested case-control designs: an application to cardiovascular disease. *Am J Epidemiol* 2012;175:715–24.
36. Coordinating Center for the Global Registry of Acute Coronary Events, Center for Outcomes Research, University of Massachusetts Medical School. Methods and formulas used to calculate the GRACE Risk Scores for patients presenting to hospital with an acute coronary syndrome. https://www.outcomes-umassmed.org/grace/files/GRACE_RiskModel_Coefficients.pdf (accessed 3 May 2016).
37. van Domburg R, Hoeks S, Kardys I, et al. Tools and techniques—statistics: how many variables are allowed in the logistic and Cox regression models? *EuroIntervention* 2014;9:1472–3.
38. Cheng JM, Garcia-Garcia HM, de Boer SPM, et al. In vivo detection of high-risk coronary plaques by radiofrequency intravascular ultrasound and cardiovascular outcome: results of the ATHEROREMO-IVUS study. *Eur Heart J* 2014;35:639–47.
39. Oemrawsingh RM, Cheng JM, Garcia-Garcia HM, et al. Near-infrared spectroscopy predicts cardiovascular outcome in patients with coronary artery disease. *J Am Coll Cardiol* 2014;64:2510–18.
40. van der Wal AC. Coronary artery pathology. *Heart* 2007;93:1484–9.
41. Rittersma SZH, van der Wal AC, Koch KT, et al. Plaque instability frequently occurs days or weeks before occlusive coronary thrombosis: a pathological thrombectomy study in primary percutaneous coronary intervention. *Circulation* 2005;111:1160–5.
42. Henriques de Gouveia R, van der Wal AC, van der Loos CM, et al. Sudden unexpected death in young adults. Discrepancies between initiation of acute plaque complications and the onset of acute coronary death. *Eur Heart J* 2002;23:1433–40.
43. Healy AM, Pickard MD, Pradhan AD, et al. Platelet expression profiling and clinical validation of myeloid-related protein-14 as a novel determinant of cardiovascular events. *Circulation* 2006;113:2278–84.
44. Yokoya K, Takatsu H, Suzuki T, et al. Process of progression of coronary artery lesions from mild or moderate stenosis to moderate or severe stenosis: a study based on four serial coronary arteriograms per year. *Circulation* 1999;100:903–9.
45. Ojio S, Takatsu H, Tanaka T, et al. Considerable time from the onset of plaque rupture and/or thrombi until the onset of acute myocardial infarction in humans: coronary angiographic findings within 1 week before the onset of infarction. *Circulation* 2000;102:2063–9.
46. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 2003;290:2292–300.
47. Bergeron N, Phan BAP, Ding Y, et al. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. *Circulation* 2015;132:1648–66.
48. Tardif JC, Tanguay JF, Wright SS, et al. Effects of the P-selectin antagonist inlacumab on myocardial damage after percutaneous coronary intervention for non-ST-segment elevation myocardial infarction: results of the SELECT-ACS trial. *J Am Coll Cardiol* 2013;61:2048–55.
49. Nidorf SM, Eikelboom JW, Budgeon CA, et al. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol* 2013;61:404–10.
50. Ridker PM. Closing the loop on inflammation and atherothrombosis: why perform the CIRT and CANTOS trials? *Trans Am Clin Climatol Assoc* 2013;124:174–90.
51. Fuster V, Sanz J. Gene therapy and stem cell therapy for cardiovascular diseases today: a model for translational research. *Nat Clin Pract Cardiovasc Med* 2007;(4 Suppl 1):S1–8.