Cohort profile of BIOMArCS: BIOMarker study to identify the Acute risk of a Coronary Syndrome, a prospective multicenter biomarker study conducted in the Netherlands

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ABSTRACT

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

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

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

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

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

Trial registration: NTR 1698 and NTR1106

INTRODUCTION

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

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

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

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

COHORT DESCRIPTION

Study objectives

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

Study cohort

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

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

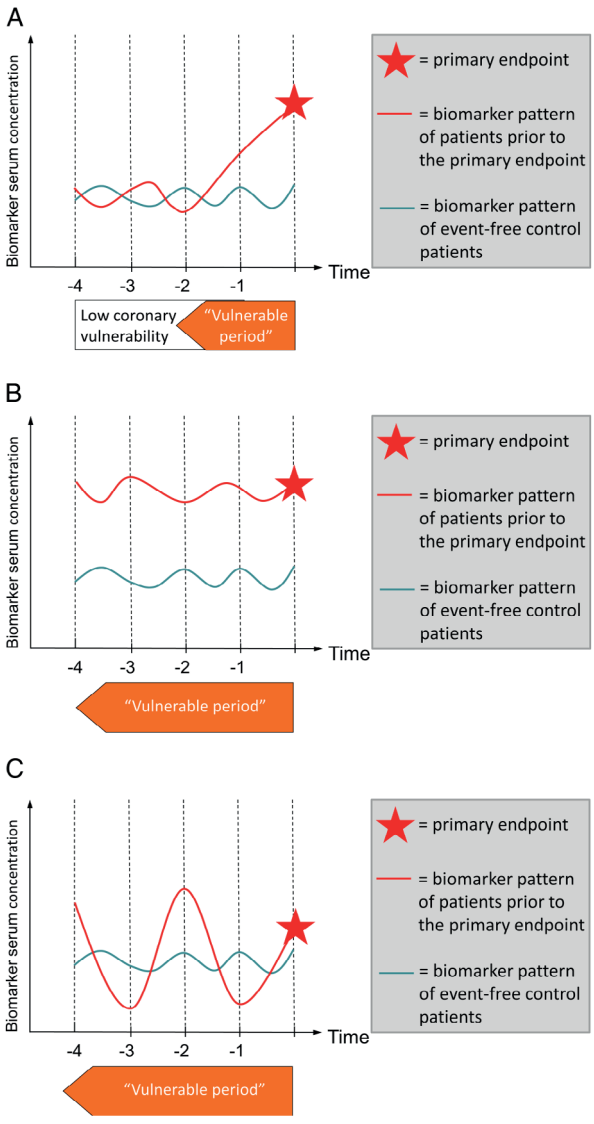

Figure 1. Different hypothetical scenarios of biomarker evolution during stable and vulnerable periods in the lifetime of a patient with coronary artery disease

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

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

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

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

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

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

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

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

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

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

Blood sample collection

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

Study endpoints

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

Sample size considerations

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

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

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

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

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

Construct of the case-cohort analysis set

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

Biomarker selection and significance testing

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

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

Table 4. Biomarker selection

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

Phase 1

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

Phase 2 (in alphabetical order)

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

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

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

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

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

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

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

Etiologic and prognostic analyses of selected biomarkers

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

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

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

Risk models

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

Early washout biomarker patterns and ancillary analyses

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

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

Study organization

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

Current status

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

DISCUSSION

Vulnerable period versus vulnerable plaque

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

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

Rationale behind the time intervals for sample collection

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

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

Future directions

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

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

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

Strengths and limitations

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

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

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

Collaboration

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

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

Contributorship statement

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

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Competing interests

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

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