High-frequency biomarker measurements of Troponin, NT-proBNP and C-Reactive Protein for prediction of new coronary events after acute coronary syndrome: The BIOMArCS Study

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

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

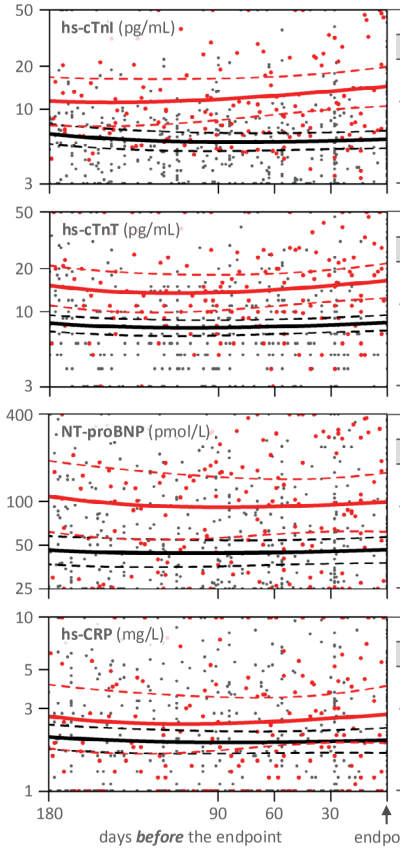
Four established CV biomarkers were then measured (in 1478 blood samples), reflecting different components of CV pathophysiology: Troponin, which was assessed with high-sensitivity cardiac Troponin I and T assays (hs-cTnI, Abbott; hs-cTnT, Roche), N-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP,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

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

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

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

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



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

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

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

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

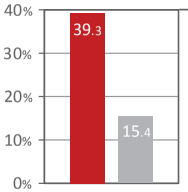
Mean 99.8 vs. 47.1 pmol/L; P-value 0.001

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

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

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

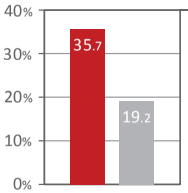
B. Percentage of cases (red) and noncases with ≥1 isolated biomarker peak value exceeding the Reference Change Value



≥1 isolated hs-cTnI value >10 pg/mL

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

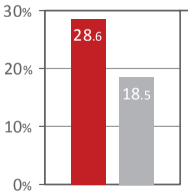
* patient-specific longitudinal hs-cTnI level



≥1 isolated hs-cTnT value >10 pg/mL

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

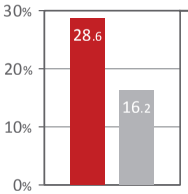
* patient-specific longitudinal hs-cTnT level



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

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

* patient-specific longitudinal NT-proBNP level



≥1 isolated hs-CRP value >10 mg/L

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

* patient-specific longitudinal hs-CRP level

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

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

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

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

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

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

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

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

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

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

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

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Conflict of Interest:

The authors have no relationships relevant to the contents of this paper to disclose.

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Study registration

The Netherlands Trial Register NTR1698

Key words

Acute Coronary Syndrome, Biomarkers, Risk Prediction

Article information

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