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

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Abstract

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

Keywords

Acute coronary syndrome; repeated measurements; myeloperoxidase; galectin-3; prognosis monitoring

INTRODUCTION

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

METHODS

Study Design

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

Case-cohort approach

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

In order to obtain detailed information on biomarker changes early after ACS admission, by design, a

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

MPO and Gal-3 measurements

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

Statistical analysis

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

Linear mixed-effect models (LME) were applied to describe the patterns of MPO and GAL-3 early after the index-ACS. We placed two splines to account for possible non-linearity. Using LME, we calculated

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

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

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

RESULTS

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

Myeloperoxidase

MPO level was elevated early after the index-ACS, with a peak value of 78.0 ng/ml at the day of admission. Within the first seven days, MPO showed a steep decline, and then stabilized after day 6 at 25.7 ng/ml (Figure 1A). During follow-up, MPO levels in cases and event-free patients were similar: after seven days, the average serum level of MPO was 26.4 (IQR: 22.1-32.4) ng/ml in cases and 25.3 (IQR: 19.9-31.9) ng/ml in endpoint-free patients. We did not observe a steady or sudden increase in MPO level prior to the reACS event (Figure 1B). The unadjusted HR for reACS per SD increase in MPO was 0.84 (95% CI 0.61-1.26) Adjustment for multiple factors did not result in a meaningful change of the estimate (Table 2).

Galectin-3

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

DISCUSSION

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

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

BIOMArCS was specifically designed to study the temporal evolution of serum biomarkers in post-ACS patients, and its highly frequent blood sampling schedule would have sufficed to identify meaningful changes in MPO and GAL-3 concentrations, had they appeared. However, contrary to our expectations, both biomarkers were not associated with an increased risk of a recurrent ischemic event during 1-year follow-up. Since the median time between the last collected sample in cases and their reACS was 11

(IQR: 5-20) days, we cannot exclude that just before reACS there might still have been biomarker elevations we did not detect. Additionally, we cannot exclude changes in MPO or GAL-3 levels during long-term follow-up.

Nonetheless, it seems that MPO and GAL-3 do not advance plaque vulnerability prior to reACS.

Conclusion

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

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Figure 1. Temporal evolution of Myeloperoxidase and Galectin 3

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