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Biomarkers

Aggregate Risk Score Based on Markers of Inflammation, Cell Stress, and Coagulation Is an Independent Predictor of Adverse Cardiovascular Outcomes

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Objectives This study sought to determine an aggregate, pathway-specific risk score for enhanced prediction of death and myocardial infarction (MI).

Background Activation of inflammatory, coagulation, and cellular stress pathways contribute to atherosclerotic plaque rupture. We hypothesized that an aggregate risk score comprised of biomarkers involved in these different pathways—high-sensitivity C-reactive protein (CRP), fibrin degradation products (FDP), and heat shock protein 70 (HSP70) levels—would be a powerful predictor of death and MI.

Methods Serum levels of CRP, FDP, and HSP70 were measured in 3,415 consecutive patients with suspected or confirmed coronary artery disease (CAD) undergoing cardiac catheterization. Survival analyses were performed with models adjusted for established risk factors.

Results Median follow-up was 2.3 years. Hazard ratios (HRs) for all-cause death and MI based on cutpoints were as follows: CRP $\geq$3.0 mg/l, HR: 1.61; HSP70 $>$0.625 ng/ml, HR; 2.26; and FDP $\geq$1.0 μg/ml, HR: 1.62 ($p < 0.0001$ for all). An aggregate biomarker score between 0 and 3 was calculated based on these cutpoints. Compared with the group with a 0 score, HRs for all-cause death and MI were 1.83, 3.46, and 4.99 for those with scores of 1, 2, and 3, respectively (p for each: $<$0.001). Annual event rates were 16.3% for the 4.2% of patients with a score of 3 compared with 2.4% in 36.4% of patients with a score of 0. The C statistic and net reclassification improved ($p < 0.0001$) with the addition of the biomarker score.

Conclusions An aggregate score based on serum levels of CRP, FDP, and HSP70 is a predictor of future risk of death and MI in patients with suspected or known CAD. (J Am Coll Cardiol 2013;62:329–37) © 2013 by the American College of Cardiology Foundation

Stable coronary artery disease (CAD) can lead to severe ischemic symptoms from stenosis-related coronary blood flow reduction, but plaque rupture leading to myocardial infarction (MI) and death is its most devastating complication. Although many patients with CAD never experience clinical plaque rupture, others may experience an early MI. Importantly, distinct genetic differences distinguish patients

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CAD = coronary artery disease
CRP = C-reactive protein
CVD = cardiovascular disease
eGFR = estimated glomerular filtration rate
FDP = fibrin degradation products
HSP70 = heat shock protein 70
IDI = integrated discrimination improvement
LVEF = left ventricular ejection fraction
MI = myocardial infarction
NRI = net reclassification improvement

with stable CAD versus those who experience plaque rupture (1). Thus, signaling pathways predisposing to atherosclerosis probably differ from those contributing to plaque vulnerability. This distinction is likely to be crucial when considering strategies for identifying patients at risk of MI and death from plaque rupture.

The Framingham Risk Score and similar scores are widely used to assess absolute risk of adverse cardiac events in patients without known CAD (2,3); however, they do not reliably predict risk of plaque rupture (and consequent MI and/or death) in patients with already established CAD (4,5). Other biomarkers appear to predict such risk, but their associated hazard ratios (HRs) have been modest (6–8). Our purpose is to develop a robust, noninvasive, and simple biomarker strategy to identify CAD patients at increased risk of plaque rupture.

The strategy we explored derived from the concept that activation of multiple pathways, including inflammatory, stress-related, and coagulation pathways, each contribute to coronary plaque instability. Elevated levels of high-sensitivity C-reactive protein (CRP) reflect vascular inflammation and are associated with greater risk for subsequent cardiovascular disease (CVD) events, but the effects are modest (9,10). Heat shock proteins (HSPs), including HSP70, are highly conserved intracellular proteins that increase in response to stress, and may provide evidence of increased cellular stress, and thus, a predisposition to plaque rupture (11–13). Both fibrinogen and fibrin degradation products (FDP), end products in the coagulation cascade, have been associated with CAD development and severity (14,15). Moreover, D-dimer, a degradation product of fibrinogen and soluble fibrin monomers, has been associated with adverse cardiac events (16,17). We hypothesized that risk assessment would be markedly enhanced when the 3 biomarkers—CRP reflecting inflammation; HSP70, associated with increased cellular stress; and FDP, associated with coagulation cascade activation—are used in aggregate, that is, the risk of plaque rupture would be greater when biomarkers reflected activation of 2 or 3 pathways compared with activation of 0 or 1 pathways.

Methods

Study population. Study participants were recruited as part of the Emory Cardiology Biobank (EMCAB), consisting of 3,763 consecutive patients enrolled before undergoing elective or emergent coronary angiograms across 3 Emory healthcare sites, between 2003 and 2009 (details in the Online Appendix).

Outcomes and follow-up. Record of death was obtained from the Social Security Death Index, and the cause of death adjudicated from medical records or direct contact was made with the patient's family member(s). Cardiac death was defined as death attributable to a cardiovascular cause or sudden death due to an unknown cause. Follow-up was conducted between 1 and 5 years to identify cases of MI and revascularization (defined as percutaneous coronary interventions or coronary artery bypass graft [CABG] surgery). MI and revascularization occurring within a month of enrollment were not included.

Identification of CAD and severity scoring. All coronary angiograms were scored for luminal narrowing using a modified American Heart Association/American College of Cardiology classification of the coronaries (18). Patients were designated as having either angiographically smooth normal coronary arteries, nonsignificant CAD (visible plaque resulting in $<$50% luminal stenosis), or significant CAD (at least 1 major epicardial vessel with $\geq$50% stenosis). Quantitative angiographic scoring was performed using the Gensini score, which quantifies CAD severity by a nonlinear points system for degree of luminal narrowing. The score has prognostic significance (19).

Sample collection. Fasting arterial blood samples for serum were drawn at cardiac catheterization and stored at -80°C (mean 4.9 years) before analysis by FirstMark, Inc. (San Diego, California) (Online Appendix). CRP and FDP levels were determined using a sandwich immunoassay. FDP components included fragments D and E, D-dimer, and additional intermediate cleavage products. HSP70 was measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) and optimized by FirstMark. Minimum detectable CRP, FDP, and HSP70 concentrations were 0.1 mg/l, 0.06 μg/ml, and 0.625 ng/ml, respectively.

Statistical analyses. Continuous variables are presented as mean ± SD, and categorical variables are presented as proportions (percentages). Student *t* test, 1-way analysis of variance, and Cochran-Mantel-Haenszel chi-square test were used as appropriate. Mann-Whitney *U* or Kruskall-Wallis nonparametric tests were performed on non-normally distributed variables. The relationship between biomarkers and outcomes was determined using the Cox proportional hazards regression in unadjusted models and in models adjusted for established risk factors that included clinically relevant covariates for CVD outcomes (age at baseline, race, diagnosis of hypertension, diabetes, dyslipidemia, use of statins, aspirin, clopidogrel, history of MI, acute MI at presentation, estimated glomerular filtration rate [eGFR; calculated using the Modification of Diet in Renal Disease equation], Gensini score, body mass index, left ventricular ejection fraction [LVEF], history of CABG, and smoking status). The proportional hazards assumption for Cox models was evaluated by plots of Schoenfeld residuals