

EDITORIAL COMMENT

Metabolomics

Seeking a Unique Biomarker Signature for Coronary Artery Syndromes*

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In this issue of the *Journal*, Bodi et al. (1) report the application of nuclear magnetic resonance (NMR) spectroscopic techniques to detect and characterize a predictive cardiac-specific metabolomic (also known as metabonomic) profile on the basis of systemic blood samples drawn from patients experiencing acute coronary syndromes (ACS) leading to myocardial tissue ischemia and infarction (i.e., acute myocardial ischemia [AMI]).

NMR spectroscopy datasets from a retrospectively selected patient population with angioplasty-induced transient AMI and a prospective clinical study design with patients with spontaneous acute chest pain are validated in a porcine model of acute and sustained coronary artery occlusion. The investigators use computational analysis of metabolomic datasets as a putative metabolic biosignature method to better inform and guide the treatment of patients presenting with symptoms of ACS.

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To obtain sensitive and specific datasets, we think it important to reflect on criteria for biomarker use in improving the diagnosis of ACS leading to AMI and subsequent tissue infarction. These criteria would include: 1) the specificity of the cardiac biomarker(s); 2) the time course of biomarker presence in the sampling space; 3) the availability of sensitive analytic methods; 4) the response time and cost of the analytic procedure; 5) the influence of medications; 6) healthy and stable angina controls; and 7) estimates of interpatient variability. Additional considerations include the appropriateness of the animal model as well as patient populations used to validate and correlate ACS with conventional ischemia-induced cardiac biomarkers.

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The term “metabolomics” was coined in 1998 by Oliver et al. (2) and Tweeddale et al. (3), while Nicholson et al. (4) coined “metabonomics” for the same concept in 1999. Metabolomics (the more common term), defining the biological response of a living system to a stimulus, involves the identification and measurement of metabolites in biological samples by either high-throughput mass spectroscopy or NMR spectroscopy. In clinical applications, a subset of metabolites associated with a particular disease is evaluated to aid diagnosis, probe pathogenesis mechanisms, and monitor treatment protocol outcomes. Early cardiovascular metabolomic studies used NMR spectroscopy to probe myocardial energy metabolism with ^{31}P resonances in adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, and phosphocreatine. Recently, ^1H NMR spectroscopy has been used in cardiac metabolomic studies, because the ^1H nucleus has high sensitivity, and molecular profile data are obtained in minutes. In the near future, it is expected that other NMR-sensitive isotopes, such as ^{13}C , will be used to identify and quantify key metabolic compounds. Terms characterizing complex metabolite analysis are evolving distinctive uses, such that metabolomics is the systematic study of the unique chemical fingerprints of specific cellular processes (most frequently used for mass spectroscopic analyses), and metabonomics is the quantitative measurement of the dynamic multiparametric metabolic responses of living systems to pathophysiological stimuli or genetic modification (most frequently associated with NMR spectroscopic analyses). Technically, Bodi et al. (1) performed a metabonomic study, looking at multiple metabolites as a function of disease state.

Predictive biomarker profiling of patients with ACS at risk for AMI leading to infarction and necrosis is a relatively recent strategy. High-sensitivity C-reactive protein and brain natriuretic peptide are prognostic biomarkers reflecting cardiovascular inflammation and heart failure, respectively. Monocyte chemoattractive protein-1 and ischemia modified albumin may also be used as biomarkers to rule out AMI and for ACS risk stratification, respectively (5).

The detection of AMI once relied entirely on aberrant biopotentials registered on electrocardiography. ST-segment elevation correlates often, but not always (i.e., “silent” ischemia, non-ST-segment elevation myocardial infarction), with myocardial ischemia (ST-segment elevation myocardial infarction) and tissue injury after coronary artery obstruction. Today, biomarkers are used in combination with the electrocardiographic signature for AMI detection.

The early search for biomarkers can be traced to the mid-20th century. More than 4 decades ago, Hess et al. (6) identified creatine kinase (CK) disorders in heart and skeletal muscle disorders, and shortly afterward, Bing et al. (7) described molecular changes in heart muscle after AMI. This was followed by the pioneering biomarker study of

Shell et al. (8), in which the peak serum concentration of CK, a rather large (43-kD monomer) intracellular myocardial protein, at approximately 12 h after coronary artery occlusion was positively correlated with myocardial infarct size in a canine model. Subsequently, from the same research group, Ahmed et al. (9) identified the CK-MB isoenzyme as a cardiac-specific biomarker for myocardial ischemia and infarction. However, Heyndrickx et al. (10), also in a canine model, measured elevated serum CK levels without myocardial infarction after brief coronary artery occlusion. During this period, Leidtke et al. (11) documented myocardial metabolic changes during coronary artery reductions in a swine model.

Today, suspected AMI is routinely evaluated by a panel of biomarkers in peripheral blood samples: myoglobin, cardiac troponin I and T, and CK-MB. Other biomarkers of myocardial ischemia include myeloperoxidase and glycogen phosphorylase isoenzyme BB.

In the context of the present study, it is important to consider relevant coronary circulation features of large animal models often used in studies of myocardial metabolism, as Bodi et al. (1) have selected a porcine model as a control. Because they evaluated patient data in retrospective and prospective study components, this animal model is a valuable calibrator and reveals relatively concordant metabolomic information after acute coronary artery ligation. Canine and porcine models are most commonly used in studies that examine facets of ACS and AMI and the accompanying changes in cardiac metabolism. The canine model, perhaps similar to the human heart with coronary artery disease and adaptive collateral vessels, is recognized for its range of myocardial ischemic responses to coronary artery occlusion due to the variable pre-existence of these coronary artery collateral vessels (12). In contrast, the porcine model, used here by Bodi et al. (1), lacks pre-existing collateral vessels and therefore is considered more similar to the healthy human heart (13).

Certainly, NMR spectroscopic capacity to detect metabolic markers combined with computational strategies for analyzing complex datasets associated with acute or chronic coronary artery syndromes is possible for the team assembled for the present study by Bodi et al. (1). Importantly, the time required to obtain results is short, making ^1H NMR metabolomic profiling for cardiovascular disease a potentially useful tool in evaluating patients in the emergency department setting. Although the major disadvantage of ^1H NMR spectroscopy is its relative insensitivity compared with mass spectroscopy, this disadvantage is usually outweighed by the low cost of sample processing and speed of data acquisition. However, the likelihood of adoption of NMR spectroscopy by first responder and community-based hospitals across the globe would seem uncertain, if only on the basis of instrumentation cost and the technical expertise required for the rapid analysis and interpretation of complex metabolic datasets. However, the cost of NMR spectrom-

eters continues to decrease and is expected to decrease more as the need to place spectrometers in hospital settings increases. This scenario was observed as the need for magnetic resonance imaging spectrometers increased as more local hospitals sought this technology. Statistical analysis of the data will become more automated, decreasing the need for highly skilled technical expertise onsite. At the same time, the method significantly advances the prognostic value of the panoply of changes in the myocardium's internal milieu when the coronary blood supply fails to meet tissue metabolic demand (14). Such an integrative approach to metabolite profile analysis as described here by Bodi et al. (1) offers substantial new opportunity for advanced biomarker patterning of what is often a rather challenging differential diagnosis of patients presenting with AMI symptoms of ACS.

In conclusion, Bodi et al. (1) provide us with compelling evidence that no single metabolite is likely to be unambiguously predictive of ischemia and altered myocardial metabolism that accompany ACS. They argue rather convincingly that an analysis of changes in the myocardial metabolic profile, using NMR spectroscopy combined with high-level biostatistical assessment of complex datasets, can supplant current best practices and thereby improve clinical decision making directing patient care for optimal outcomes. One can only hope such advances will reduce the frequency of both false-positive and false-negative findings arising from the use of current biomarkers.

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