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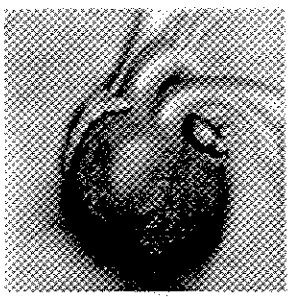
An Analysis of Cardiovascular Biomarkers

Biomarker analysis plays an integral role in modern medicine.

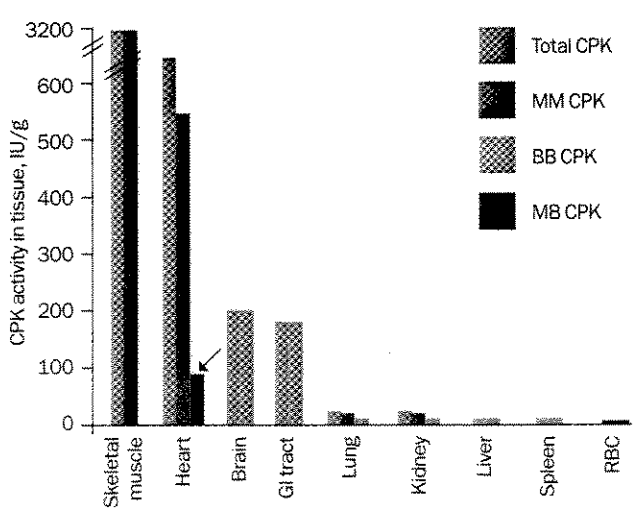


By Alex Harrison, MD; Howard Kirchick, PhD; Scott Mader; and Barbara M. Goldsmith, PhD, FACB

The term "biomarker" refers to a specific biochemical within the human body that has a particular molecular feature that makes its measurement useful for assessing the progress of disease, effect of treatment and / or offers prognostic information. As technology and molecular biochemistry continue to advance, more of these biochemicals are discovered and their application as potential clinical biomarkers is investigated.

Over time, biomarkers have played an integral role in the practice of cardiovascular medicine. Their expanding use in this arena can be used as a microcosm for their use and growth throughout medicine. An in-depth analysis surrounding the historical, current and future applications of biomarkers within cardiovascular medicine allows a detailed examination that can then be more generally applied to medicine as a whole.

Fig. 1
Distribution of CK Activity



Distribution of creatine kinase MM, BB and MB activity in different organ systems. Note the lack of substantial CK-MB activity (blue columns) in all organs but the heart (arrow). *(Data from Roberts R, Gowda KS, Ludbrook PA, Sobel BE.* Am J Cardiol *1975;36:433.)*

Evolution of Single Biomarkers

Myocardial Necrosis

The use of biomarkers to detect an acute myocardial infarction (AMI) is the standard of care. However, the biomarkers for detecting myocardial necrosis evolved over time. In the 1950s, lactate dehydrogenase (LDH) became the first biomarker clinically utilized in efforts to detect myocardial necrosis during a myocardial infarction. LDH consists of M (muscle) and H (heart) subunits that give rise to five isoenzymes. The heart primarily contains LDH-1 and some LDH-2. Red cells, kidney, stomach and pancreas are other important sources of LDH-1.

In contrast, LDH-5 predominates in skeletal muscle and liver.[1,2] Serum LDH activity rises to abnormal levels approximately 10 hours after the onset of an AMI, peaks at 24-48 hours, and remains elevated for six to eight days. However, the lack of specificity for the heart led to the use of other biomarkers to detect myocardial necrosis.

The next biomarker utilized to detect myocardial necrosis »

was creatine kinase (CK). While this biomarker offered some advantages over LDH, it, too, was limited because of its wide distribution throughout the body and lack of specificity to heart tissues.

However, isoforms of the CK isoenzymes were characterized and helped bring more specificity to detect cardiac myocyte necrosis. CK isoenzymes are dimers of M and B chains and exist in three combinations: MM, MB and BB.[3] These isoenzymes reside in the cytosol and facilitate the egress of high energy phosphates into and out of mitochondria.[4]

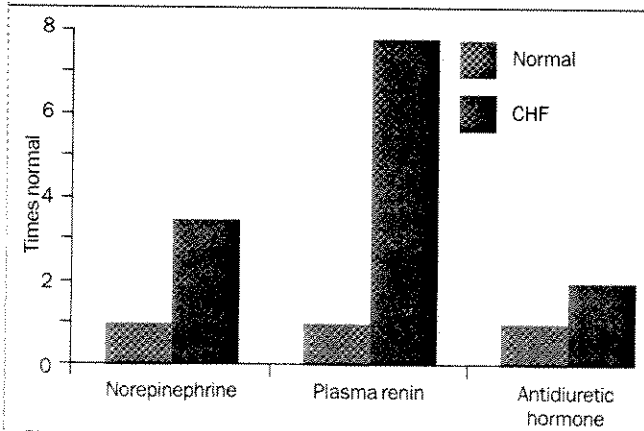
CK isoenzyme activity is distributed in a number of tissues; the CK-MB fraction on a percentage basis is generally confined to heart tissue (Fig. 1).[5] CK-MB has high specificity for cardiac tissue and is still widely utilized as a marker of cardiac injury to help diagnose an AMI.[6,7] As with total CK, CK-MB typically begins to rise four to six hours after the onset of infarction but is not elevated in all patients until about 12 hours.

In the 1990s the discovery of cardiac troponins added to the biomarkers specific to cardiac tissue. Cardiac troponin I (cTnI) and T (cTnT) are cardiac regulatory proteins that control the calcium-mediated interaction of actin and myosin.[8]

Table
Potential Novel Biomarker Risk Factors for Cardiovascular Disease

Markers of Inflammation	Markers of Hypercoagulability
High sensitivity C reactive protein (hs-CRP)	Hyperhomocysteinemia
Serum amyloid A (SAA)	Fibrinogen
Fibrinogen	Activated protein C resistance
Myeloperoxidase (MPO)	von Willebrand factor antigen
Interleukin-6 (IL-6)	Coagulation factors VII and VIII
Tumor necrosis factor alpha (TNF-α)	Prothrombin fragment 1.2
Intercellular adhesion molecule (ICAM-1)	Markers of Impaired Fibrinolysis
Vascular adhesion molecule (VCAM-1)	Plasminogen activator inhibitor (PAI-1)
E selectin and P selectin	Tissue type plasminogen activator (t-PA)
	D-dimer
	Clot lysis time
	Lipoprotein (a)

Fig. 2
Hormone Levels in CHF



Plasma levels of norepinephrine, renin activity and antidiuretic hormone are increased two- to eight-fold (when compared to normal subjects) in patients with stable congestive heart failure treated with digitalis, but not diuretics or vasodilators. *(Data from Francis GS, Goldsmith SR, Levine TB, et al.* Ann Intern *1984; 101:370.)*

Cardiac troponin concentrations usually begin to rise four to six hours after an MI, a time course similar to that with CK-MB. Elevations in serum cTnT and cTnI reflect small areas of myocardial necrosis in patients with unstable angina or non-ST-segment MIs and also predict the severity of disease. This was illustrated in the FRISC II trial in which any elevation of cTnT in the noninvasive arm of the study increased the likelihood of severe three vessel disease, an unstable plaque with thrombus and downstream microembolization, and impairment of coronary flow. These factors are all associated with an increased risk for reinfarction and death.[9] The degree of elevation of cTnI or cTnT also has prognostic value as illustrated by the GUSTO IV ACS trial, the TIMI IIIB trial, the GUSTO IIa trial and FRISC study.[10-14]

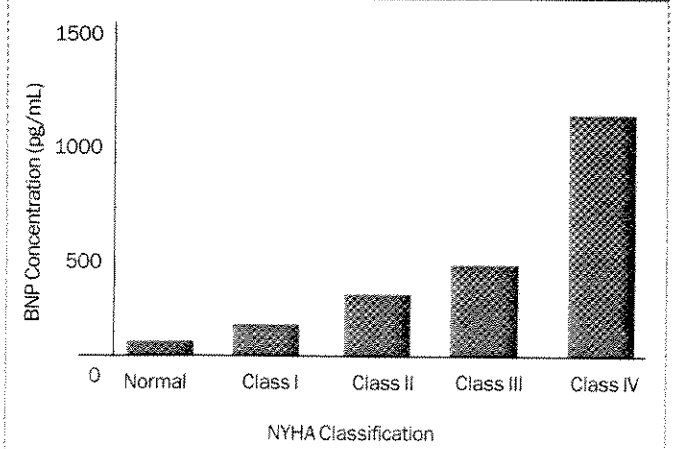
Cardiac Neurohormonal Activity

Initially used to assist in the diagnosis of myocardial necrosis, the use of cardiac biomarkers has broadened to help clinicians understand the neurohormonal activity of the cardiovascular system. When the heart begins to fail to provide the same cardiac output it once had, the body compensates to maintain perfusion. The signs and symptoms of heart failure (HF) are due in part to compensatory mechanisms utilized by the body in an attempt to adjust for this primary deficit in cardiac output. Neurohumoral adaptations, such as activation of the renin-angiotensin-aldosterone and sympathetic nervous systems by the low output state, can contribute to maintenance of perfusion of vital organs in two ways:

1. maintenance of systemic pressure by vasoconstriction, resulting in redistribution of blood flow to vital organs, and
2. restoration of cardiac output by increasing myocardial contractility and heart rate and by expansion of the extracellular fluid volume.[15,16]

Specific biomarkers can be analyzed to help clinicians assess the degree of this neurohormonal activation of the renin-angiotensin-aldosterone and sympathetic nervous system. In the short term, neurohumoral activation is beneficial in patients with HF since elevations in cardiac contractility, vascular resistance and renal sodium retention tend to restore the cardiac output and tissue perfusion toward normal. However, the deleterious effects may predominate over the long term, leading to pulmonary and peripheral edema, increased afterload, pathologic myocardial remodeling and more rapid progression ▸

Fig. 3
Median BNP Concentrations vs. NYHA Classification*



*DATA FROM BIOSITE INC.

Fig. 4
Cardiac Marker Temporal Patterns

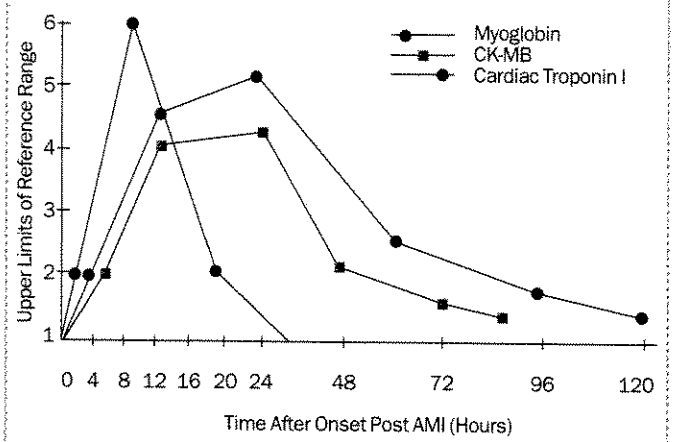


Fig. 4 shows the temporal pattern of cardiac biomarker release after the onset of an acute myocardial infarction (AMI). The three markers illustrated are myoglobin, CK-MB and cardiac troponin I (cTnI). The y-axis shows the relative elevation above the upper limit of reference of each biomarker. The x-axis demonstrates the time after AMI measured in hours. The figure illustrates that serum myoglobin is elevated most rapidly after the onset of an AMI and that cTnI remains elevated for the greatest duration of time post AMI.

of myocardial dysfunction. The ability of ACE inhibitors and beta blockers to improve survival and slow the progression of the heart failure corroborate this pathophysiological occurrence.

Biomarkers have proven to help clinicians assess this degree of neurohormonal activation. Measurements of plasma norepinephrine, renin and antidiuretic hormone have proven to be significantly elevated in patients with HF (Fig. 2). Through years of research, b-type natriuretic peptide (BNP) has proven to be the most stable, consistent and reliable measurement of the degree and severity of heart failure.[17,18] The correlation between BNP levels and New York Heart Association Class is shown in Fig. 3. Aside from diagnosing CHF and assessing the degree of neurohormonal activation, BNP measurements have proven valuable in:

- monitoring patients,
- tailoring management and titrating therapy,
- providing objectivity in assessing discharge and admission criteria,
- predicting adverse cardiac events and readmissions in CHF inpatients and
- offering overall prognostic information.[19-23]

Risk Factors for Coronary Disease

Cardiac biomarkers also have found tremendous and growing utility in assessing risk factors and markers of coronary heart disease (CHD). Current cardiovascular risk assessment utilizes the Framingham database that identifies the following cardiovascular risk factors:

1. those unable to be changed—age, gender, heredity—and
2. those that can be changed—blood pressure, cigarette smoking, obesity, glucose intolerance / diabetes, stress and cholesterol.[24]

There is growing data, however, surrounding the role of inflammation, hypercoagulability and impaired fibrinolysis in the pathogenesis of coronary atherosclerosis. Potential biomarkers under investigation include those illustrated in the Table.[25-28] Numerous studies have indicated the potential role these biomarkers may play in future of cardiovascular risk analysis.[25-28]

Transitioning to Biomarker Panels

As new and more specific biomarkers are discovered, the best approach to incorporate them into clinical practice becomes challenging. More recent discoveries are proving to be complementary, as opposed to superior, to previous markers. Unlike earlier developments with cardiac necrosis markers where there was a linear progression from LDH to CK to CK-MB, there has been a vast expansion in available biomarkers with various implications and potential applications. The use of cardiac biomarkers in the setting of an acute coronary syndrome (ACS) is moving toward a multimarker strategy.

While serum troponins and CK-MB detect myocardial cell death, other less specific but highly sensitive markers detect myocardial cell injury sooner. Serum myoglobin, while currently underutilized, has tremendous potential for early detection of myocardial infarction. Among patients with an AMI, the serum myoglobin concentration is elevated in roughly similar proportions as CK-MB and troponins.[29] The delayed release of the cTnI and CK-MB decreases their sensitivity until four to six hours after the onset of symptoms (Fig. 4); some patients do not show an enzyme elevation for as long as 12 hours. Serum myoglobin rises within one to four hours and is more sensitive during this early period than the other markers.[30] The CHECKMATE study showed that an approach utilizing rapid multimarker analysis identifies patients with an AMI earlier, and provided better risk stratification for mortality than a local laboratory-based, single-marker approach.[31]

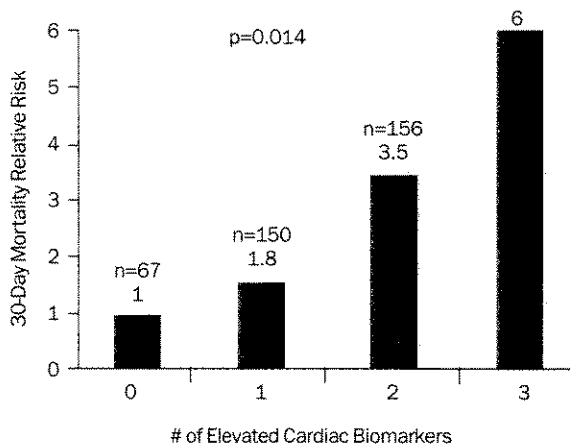
Cardiac troponin concentrations usually begin to rise four to six hours after an MI, a time course similar to that with CK-MB (Fig. 4). However, at least 12 hours is required to detect elevations in all patients with both cTnI and CK-MB. As a result, serial testing is performed after four or more hours if the initial values are indeterminate, the ECG is not diagnostic, and clinical suspicion of an MI remains high. Elevations in serum cTnI levels after AMI persist for up to 10 days, permitting late diagnosis.[29,32]

CK-MB has high specificity for cardiac tissue and is still commonly utilized for detecting AMI.[29] As with total CK, CK-MB typically begins to rise four to six hours after the onset of infarction but is not elevated in all patients until about 12 hours (Fig. 4). An elevated serum CK-MB is relatively specific for myocardial injury, particularly in patients with ischemic symptoms when skeletal muscle damage is not present. These elevations return to baseline within 36-48 hours compared to durations as long as 10 days seen with troponins. Thus, CK-MB cannot be used for late diagnosis, but new elevations can help detect infarct extension.

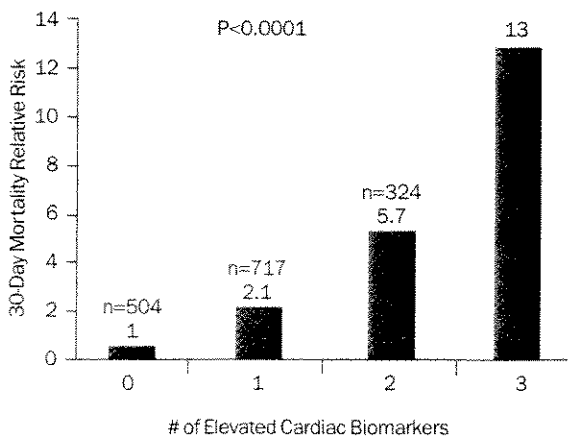
An elevation in the serum concentration of one or more of the above markers (myoglobin, CK-MB, cTnI) is seen in virtually all patients with an AMI.[31,32] The Diagnostic Marker Cooperative Study, a large, prospective, double-blind study of patients who presented to the emergency department with chest pain, showed that CK-MB subforms (91 percent and 89 percent) and myoglobin (78 percent and 89

Fig. 5

Relative 30-Day Mortality Risks in OPUS-TIMI 16 in Patients Stratified by Number of Elevated Cardiac Biomarkers



Relative 30-Day Mortality Risks in TACTICS-TIMI 18 in Patients Stratified by Number of Elevated Cardiac Biomarkers



INFORMATION ADAPTED FROM SABATINE M, ET AL. *CIRCULATION* 2002;105:1760-63.

percent) were most sensitive and specific within six hours of symptom onset, while total CK-MB (96 percent and 98 percent) and troponins (96 percent and 93 percent for cTnI) were most sensitive and specific at 10 hours.[31] To date, serial analysis of these three biomarkers to assess cardiac myocyte necrosis is the most sensitive and specific method to detect an AMI.

Through meta-analysis, Sabatine et al[33] showed that a multimarker approach to risk stratification in non-ST elevation acute coronary syndromes is extremely valuable. A simultaneous analysis of cTnI, C-reactive protein (CRP) and BNP provides unique prognostic information where each marker has independent and incremental prognostic information. The 30-day risk of death increased in proportion to the number of cardiac biomarkers elevated at baseline (P=0.014), with a near doubling of the mortality risk for each additional biomarker that was elevated (Fig. 5). Similar relationships existed for the endpoints of MI, CHF and the composite, with persistent predictive capacity through 10 months.[33]

As new necrosis markers are discovered with varying favorable characteristics—improved sensitivity, specificity or earlier allowable detection—the new markers will likely be incorporated into a multimarker approach to capture additional information rather than supplanting the existing markers. Newer biomarkers such as myeloperoxidase (MPO) and ischemia modified albumin (IMA) are such potential future necrosis markers.[34,35] A similar multimarker approach is gradually finding its way to other areas of cardiovascular biomarker implementation. The use of BNP is expanding tremendously from its initial use of diagnosing CHF in the emergency department, and now has found use in monitoring progression of HF, titrating treatment and assessing overall prognosis.[19-23] With its expanding utilization, BNP is becoming incorporated into many biomarker analyses.

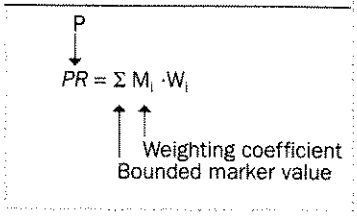
Panel Response Biomarker Testing

The future of biomarker analysis appears to be heading toward the technological advances in high-throughput robotic workstations and software applications that can assimilate biomarkers into a single "derived analyte." The concept thrives on the technological advances in biotechnology that allow the analysis of dozens to even hundreds of biomarkers in search for those that contribute significant information about a desired analysis. The biomarkers that offer such information are then combined into a panel that sums their respective information into a panel response or single-derived analyte.

Fig. 6 illustrates this concept, where the panel response (PR) equals the sum of each biomarker's product of the measured biomarker value (M) multiplied by a weighting coefficient (W). To prevent any single biomarker from overweighing the summation, each (M) is restricted by an upper and lower limit. Values above and below the limits are set to the limits. This panel response analyte can then be used in the same fashion as individual markers where receiver operated characteristic (ROC) curves can be generated and offer cutoffs with optimal sensitivity, specificity and accuracy.

This technology, referred to as panel response biomarker testing, is pioneering its clinical utility in the area of stroke and vascular injury, with applications in other areas close behind. A panel response system has been developed to help clinicians identify patients with an ischemic stroke. Given the absence of a widely available and sensitive means to objectively identify such patients, a panel response that could accurately identify cerebral ischemia would be a valuable clinical tool. Greater than 50 biomarkers involved in the ischemic cascade of glial activation, ischemic neuronal injury, inflammation and markers of impaired hemostasis and thrombosis were analyzed; the best indicators of cerebral ischemia were combined into a derived analyte or panel response that provided a sensitivity and specificity of greater than 90 percent for detecting stroke.[36,37]

Fig. 6
Panel Response Concept



This technology could potentially allow the simultaneous analysis of all cardiovascular biomarker risk factors detailed in the Table and provide a single derived analyte with tremendous noninvasive insight into the current status of a person's cardiovascular system and CAD risk. This distilled cumulative analysis could have tremendous clinical significance and serve to guide future therapeutic and risk modification strategies.

Similarly, a panel response system developed for ACS could help detect AMIs earlier, help risk stratify those at tremendously high risk for an MI but who don't have active myocardial necrosis. In addition, it could help identify individuals with an MI who have significant neurohormonal activation of the renin-angiotensin-aldosterone and sympathetic nervous system and are at increased risk of developing heart failure. ■

Dr. Harrison is with the Division of Medical Education and General Internal Medicine, Scripps Mercy Hospital, San Diego; Dr. Kirchick is employed by Biosite Inc., San Diego; Scott Mader is the founder of Clindevor 360 Inc., San Diego; and Dr. Goldsmith is executive director, Lab Alliance, Cincinnati.