

Looking high and low for cardiac markers



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The tale of an ailing heart is told in a biochemical language that a multi-marker panel may someday be able to translate into a better diagnostic, prognostic, and treatment roadmap than now exists.

“Clinicians say they want [cardiac] biomarkers to do three things: Define the disease, reflect the potential underlying etiology, and help guide therapy,” said Robert Christenson, PhD, in a recent American Association for Clinical Chemistry audioconference, “Cardiobiomarkers: New Applications and Multimarker Strategies.” Researchers are testing several new cardiac biomarkers and gaining new insight into how to use existing ones to achieve those goals.

Cardiac troponin is the cornerstone now for ruling in acute myocardial infarction, said Dr. Christenson, a professor of pathology at the University of Maryland School of Medicine, Baltimore. And understanding how this cardiac necrosis marker works and its shortcomings provides a basis for using additional biomarkers to speed diagnosis and treatment of acute coronary syndromes, or ACS.

One can think of ACS as occurring on a continuum of ischemia ranging from “green to red” where green represents a “bit of unstable plaque causing a little clotting that is mostly resolved by the body’s natural defenses,” Dr. Christenson says. And red is a “rip snorting ST-segment elevation myocardial infarction or sudden death MI.”

In detecting ACS, cardiac troponin “is an exquisite marker” because elevated levels virtually always translate to a myocardial source, Dr. Christenson says. And it’s rarely falsely positive, though that can occur due to analytical problems, he adds.

The draft National Academy of Clinical Biochemistry guidelines for biomarkers of ACS and heart failure say that in patients with a clinical syndrome consistent with ACS, a maximal (peak) concentration exceeding the 99th percentile of values (with acceptable precision) for a reference control group on at least one occasion during the 24 hours after the clinical event is indicative of MI.

Because of problems with standardization, patients should at this point “optimally have their troponin I done by the same method or lab,” Dr. Christenson says. But the AACC troponin I standardization subcommittee is winding up its work now, he says, the goal of which is to harmonize troponin I results much like total cholesterol or glucose measurements are harmonized. “Then you can develop a

cutoff for a positive troponin in evaluating patients for [cardiac] tissue death at perhaps the 99th percentile of the reference control population, and this will be virtually the same for all technologies.” (Standardization and harmonization of troponin T hasn’t been an issue since only one assay manufacturer makes it, according to the draft NACB guidelines.)

Troponin in general rises about six to eight hours after an acute MI. In patients presenting with signs of ACS, clinicians should “follow the temporal sequence and draw [blood for testing] at presentation, at six to nine hours and 12 to 24 hours,” Dr. Christenson said in his presentation.

Obtaining sequential troponin values identifies patients with MI who initially test negative for cardiac necrosis. But it can also help rule out MI in patients with initially elevated troponin due to another cause of cardiac injury, such as myocarditis. Initially elevated troponin levels in a patient with myocarditis should remain about the same six to nine hours later, Dr. Christenson says.

Troponin must be interpreted within the clinical context, he stresses. In that regard, it’s a bit like human chorionic gonadotropin. If you hear someone has an elevated HCG in the ED, you’re going to “think pregnancy.” But when you see the patient is a “45-year-old male with a testicular mass, the picture changes,” he notes. Similarly, when the troponin level is elevated, physicians tend to think heart attack, though the necrosis marker simply measures cardiac cell injury.

Cardiologist Christopher deFilippi, MD, who co-presented the AACC audioconference, noted that patients with end-stage renal disease on dialysis tend to have chronically elevated troponin levels, especially troponin T which doesn’t easily clear the dialysis membrane. “The thinking is that a patient on dialysis will have thickening of the heart muscle and the inner portion of the heart may not get adequate blood flow, leading to injury or death,” though no one is quite sure, says Dr. deFilippi, associate professor of medicine, Maryland Heart Center, University of Maryland. Thus, doing serial testing on dialysis patients when they present with signs of ACS should be considered the norm, Dr. deFilippi says. You’d expect to see an increase in troponin from the baseline if the patient had suffered acute MI, he adds.

Evidence shows that a positive troponin can guide treatment to improve outcomes for patients with non-ST-segment elevation myocardial infarction at presentation who thus require troponin testing to diagnose MI. A meta-analysis of all major randomized clinical trials shows that use of glycoprotein IIb/IIIa inhibitors in that population can reduce by 15 percent death/MI at 30 days, Dr. Christenson said in his AACC presentation ([Lancet](#) 2002;359:189–198).

Adding the natriuretic peptides to troponin testing can provide separate information about the impact of a patient’s ACS on his or her heart. Troponin is a necrosis marker that shows cell death. By contrast, the hormonal markers, B-type natriuretic peptide, or BNP, and the metabolite N-terminal portion of pro-BNP (NT-pro-BNP), show the heart has undergone hemodynamic stress, which can be caused by ischemia or necrosis.

According to some data in the literature, Dr. Christenson says, “if a patient has a positive troponin and BNP over, say, about 80 pg/mL, he or she is at particularly high risk” of adverse outcomes. “That is a simple example of someone whom the

American Heart Association/ American College of Cardiology guidelines indicate may benefit from intervention including an anti-platelet drug.”

Experts agree you can’t get much better than troponin at detecting cardiac necrosis. But having to wait six to eight hours for troponin to turn positive impedes early intervention.

Some emergent-care settings thus use CK-MB, which rises about an hour before troponin turns positive, or they may use myoglobin, a necrosis marker that rises about one to three hours post-MI.

Myoglobin is a popular test in Europe, Dr. Christenson says. “But many clinicians in the U.S. feel like it may be a misleading test because, unlike the cardiac troponins, myoglobin is released from both skeletal and cardiac muscle. And it’s been speculated that about 25 percent of patients presenting to emergency rooms will have elevated myoglobin from skeletal muscle injury, renal disease, or other causes,” he notes.

Yet despite myoglobin’s nonspecificity, “it may be included as a rule-out test in early assessment,” Dr. Christenson adds.

Cardiologist David Morrow, MD, MPH, says, “Some data say use of myoglobin improves prediction of mortality in ACS” though it has a lower specificity than troponin. Dr. Morrow, who also was a speaker in the AACC audioconference, is with the cardiovascular division and the Thrombolysis in Myocardial Infarction (TIMI) Study Group at Brigham and Women’s Hospital, Boston.

Patients presenting in the ED with chest pain or other symptoms of ACS who receive negative serial troponin results are hardly home free. Dr. Christenson notes that, according to the “classic data” looking at “a pie chart of all the people who present in the ED with ACS,” about 40 percent of them will have disease in part of the ACS continuum that troponin can’t detect.

Thus, the hunt continues for ischemia markers to identify patients who could benefit from intervention to stave off impending MI or reduce their risk of having one in the short-term.

In that regard, myeloperoxidase, or MPO, is promising as a biomarker that can identify troponin-negative patients at risk for MI, says Dr. deFilippi. In August 2005, the Food and Drug Administration approved the first MPO assay, CardioMPO, which was developed by Prognostix, a spin-off of the Cleveland Clinic Foundation. The assay is cleared for use in conjunction with clinical history, electrocardiogram, and other cardiac biomarkers to evaluate patients with chest pain at risk for major adverse cardiac events.

MPO appears to provide independent information separate from BNP and troponin, Dr. Christenson says. It’s involved in the inflammatory process and is therefore “elevated in response to physiological perturbations such as cardiac ischemia.” Neutrophils and monocytes, “which are loaded with MPO, are recruited to the injured tissue where MPO is released when plaque is unstable or in the event of ruptured plaque and cell injury.”

MPO works based on the premise that you can identify patients with chest pain

who are at risk of MI but don't have a positive troponin or EKG changes, Dr. deFilippi says. In cases where a patient had an elevated MPO, "the physician...would probably order some sort of functional study to see if exercise or medication to simulate exercise detects evidence of ischemia."

MPO "is a very early predictor of cardiac risks, even in patients who present within four hours of onset of chest pain," says Stanley Hazen, MD, PhD, section head of preventive cardiology and cardiac rehabilitation at the Cleveland Clinic Foundation and the senior investigator of a study on MPO ([*N Engl J Med*. 2003;349:1595–1604](#)). Dr. Hazen is also the scientific founder of Prognostix.

"Studies submitted to the FDA—similar to the one we published in the *New England Journal*—show that in patients who present with a history of chest pain, an elevated MPO level increased the risk of MI at presentation or subsequent risk for MI, need for revascularization, or death over the ensuing one-month and six-month intervals," Dr. Hazen says. Most important, he adds, high levels of MPO predicted those negative outcomes in individuals who "ruled out" for an MI at presentation—that is, the group of patients persistently negative for troponin or CK-MB.

In fact, the study data show that use of serial troponins as the only risk-stratification screen identified 89 percent of subjects presenting with signs and symptoms of ACS who ultimately experienced a major adverse cardiac outcome (for example, MI, a revascularization procedure, or death) within one or six months of presentation, Dr. Hazen adds. That means troponin missed 11 percent of the at-risk patients. But "adding MPO to the risk-stratification screen by using a low-level cutoff to define low-risk subjects markedly reduced the number of missed subjects to less than one percent..." Alternatively, using a high-level cutoff substantially improved the ability to identify subjects at increased risk for a major adverse cardiac event over the next 30-day and six-month interval, Dr. Hazen says.

The researchers obtained only initial MPOs on study participants. Now that several hospitals are using MPO testing, the question has come up about what additional information serial MPO values might provide, Dr. Hazen says. For example, the Cleveland Clinic, which has had MPO testing available for several months now, has found that MPO levels can change rapidly (the findings aren't part of a published study).

"The [MPO] levels can go up in a couple of hours and come down in a matter of a few hours," Dr. Hazen says. "How the MPO levels change depends on the individual patient; some patients have levels that continue to rise, which you often see with ST segment elevation MI. Other patients' MPO comes down at a variable rate—either slowly or within a few hours."

For now, he says, "it is probably prudent to consider any high MPO value as [placing the patient] at risk, if looking at MPO with serial cardiac enzymes."

Thus far, the MPO levels typically seen in people at risk for major adverse cardiac events have been much higher than in people with infectious or other inflammatory disorders, Dr. Hazen says. "So while we see people with sepsis have MPO levels on the higher side or upper end of normal cutoffs, the levels of MPO for people at high risk for major adverse cardiac outcome or ACS are three to four times the upper limit of normal."

The absolute values of MPO reported in many future published studies are going to be much higher than in previously published ones, which used research-grade assays with low recovery of MPO, Dr. Hazen says. “Prognostix, which made the first FDA-cleared immunoassay for in vitro diagnostic use, is serving as a gold standard for the other diagnostic companies developing MPO testing,” he adds. “Units have been highly variable from one publication to another, but there is now an agreement to follow Prognostix’s lead and use the same units of measurement.”

Biosite Inc. has applied to the FDA for clearance of a point-of-care MPO test. And Abbott and Dade Behring will come out with high-throughput platforms in a couple of years, Dr. Hazen reports. “All are using CardioMPO as the gold standard, sharing standards, calibrants, and performing cross-validation studies in an effort to match results across platforms. We’re trying to avoid what happened with troponin I, or even BNP, where tests run on alternative platforms have different ranges reported.”

Biosite plans to position MPO in two ways. One is to offer it as a standalone point-of-care test with the same FDA-approved indications that CardioMPO now has. “The POC test could be used in physician offices but most likely will be used in the ED,” says Kenneth Buechler, PhD, president, chief scientific officer, and co-founder of Biosite. “The goal of our product is to define ACS with the very first draw so that subsequent draws, which now are standard practice, for the diagnosis would not be necessary.”

Biosite also plans to add MPO to its Triage CardioProfiler, a panel of markers that already includes troponin I and its complexes, CK-MB, myoglobin, and BNP.

MPO isn’t the first FDA-cleared ischemia marker. Ischemia-modified albumin, or IMA, developed by Ischemia Technologies, was first out of the gate in 2003 with promise that it could rule out cardiac ischemia in people at low risk for ACS. In clinical practice, however, large numbers of patients test positive for IMA in the ED setting. “There may be a problem with the assay or with the marker itself,” says Dr. deFilippi, who suspects the issue may be a lack of specificity.

But the “story for IMA” may not be over yet, predicts Alan Wu, PhD, professor of laboratory medicine at the University of California at San Francisco. He notes that Ischemia Technologies is under new ownership “and we may see IMA be released in some other form.”

Dr. Wu says there are about a dozen cardiac biomarkers under study, including choline and pregnancy-associated plasma protein A. “Which ones will survive will be determined in the next several years,” he says.

Among the candidates: Soluble CD40 ligand (sCD40L), a marker of platelet activation and inflammation. “Repeated studies show that the baseline level of sCD40L in ACS is associated with clinical prognosis,” Dr. Morrow says.

A research study in the past year has renewed interest in heart-type fatty acid binding protein (H-FABP) as a necrosis marker that may have better cardiac specificity than myoglobin, Dr. Morrow says. Like myoglobin, the marker rises early, or one to three hours after onset of symptoms of ACS.

C-reactive protein, a nonspecific inflammatory marker, has “strong data” with

more than 12 studies “linking it to prognosis” in the setting of ACS, stable coronary artery disease, and patients at risk for coronary artery disease, Dr. Morrow says. But “treating clinicians are still trying to figure out the implications of what to do with data showing that elevated CRP is strongly associated with prognosis in the setting of [those conditions], especially the risk for mortality.”

However, there is evidence, he adds, that statins lower CRP and may reduce the [cardiac] risk associated with higher levels. “At least one study testing a statin for patients at risk for CAD who have elevated CRP but below average cholesterol levels is underway,” he reports.

Why might CRP flag risk of ACS? One explanation: A global body inflammatory response “sets a person up for ACS,” says Dr. Christenson. “There’s that notion of ‘bad blood,’ as our grandmothers used to say.” And that may be the widespread inflammation that primes the platelets “so they are accidents waiting to happen,” he says. And if a person happens to have a cardiac issue and his or her platelets are “ready for action” this person “may have an increased propensity for thrombus formation and consolidation that might have otherwise been dissolved by a person’s native mechanisms.”

Research continues to shed light on using cardiac biomarkers in the ambulatory-care setting to detect a person’s risk for developing heart failure. An article published in the January 2006 *Journal of the American College of Cardiology* found that “BNP—and particularly the N-terminal portion of pro-BNP—was importantly sensitive in detecting ventricular dysfunction in the general population, especially in people 65 and older,” says the article’s lead author, John Burnett, MD, a cardiologist and director of the Cardiovascular Research Center at the Mayo Clinic, Rochester, Minn. The finding proved to be true especially in males but less so in females. Dr. Burnett says researchers don’t know quite how to explain that finding. But females tend to have a higher level of the biomarker even under normal conditions, he says. “Thus the greater range of the peptide may make the biomarker a little less sensitive.”

The study involved a general population of 2,000 randomly sampled individuals age 45 and older. Some had a past history of MI, hypertension, and diabetes, Dr. Burnett says. “Yet the goal of the study was not to understand the potential cause of why people had elevated BNP; it was to look at whether these cardiac biomarkers can detect left ventricular dysfunction in the general population,” he explains.

In a followup study to be published soon in *Hypertension*, Dr. Burnett and his co-workers looked at the same population but excluded all people with documented heart failure. “We have now followed these people for seven years,” says Dr. Burnett, “and found that those age 45 and above who have a BNP in the upper third of the normal range of BNP for the general population had an increased risk of death within seven years.” The study didn’t determine the cause of death.

Dr. Burnett points out that a BNP in the upper third of the range is not BNP in the heart failure range. “But we found through echocardiography that [people with that level of elevated BNP] did have underlying hypertrophy of the heart and other structural changes,” he says. “And when we looked at risks, that population indeed had a higher incidence of coronary artery disease, hypertension, and other risk factors.”

Dr. Burnett's group presented the study findings at the American College of Cardiology annual meeting in March. "The sense at the ACC meeting was that the study had major implications for the general population and for a potential strategy utilizing BNP to identify people at risk who need better risk factor modification and therapy," he says.

"The study findings tell us that the biomarkers, BNP and N-terminal portion of pro-BNP, can be used not only to detect heart failure but also in primary prevention," Dr. Burnett says. Applying the study findings, a clinician who saw a patient age 45 or older with a BNP in that upper range of normal could refer the patient for additional workup. "If further testing identified a structure change of the heart or other risk factors, the clinician could aggressively manage the person," he says.

The primary intervention now would be more aggressive control of risks, such as hypertension and hyperlipidemia. Studies show that certain drugs like ACE inhibitors and angiotensin receptor blockers can reverse ventricular hypertrophy. And when that occurs, the BNP levels decline, he says.

Clinicians could also use BNP levels in the upper range of normal as the "the canary in the mine" to identify patients who should be taught to avoid nonsteroidal inflammatory drugs, Dr. Burnett says. NSAIDs have a tendency to precipitate and exacerbate heart failure—a threat Dr. Burnett says he's surprised that clinicians don't take more seriously.

The Mayo Clinic researchers plan to recommend that the National Institutes of Health conduct a large primary prevention trial that would use available therapies for primary prevention more aggressively to treat patients with BNP levels in the upper range of normal. "Our hypothesis is that the ability to see the BNP level and continuously try to aggressively lower it would produce a survival benefit over the lack of that information for" a control group whose cardiovascular risks were managed aggressively without the BNP testing, Dr. Burnett says.

Evidence also exists that MPO may help pick up left ventricular dysfunction, says the Cleveland Clinic's Dr. Hazen ([*Arterioscler Thromb Vasc Biol.* 2005;25:1102–1111](#)). As a potential explanation, he notes that "in animal models, when we knock out the gene for MPO, and induce a heart attack, the animal does so much better in terms of developing less heart failure because it doesn't have MPO on board. The MPO in white blood cells plays a role in promoting tissue damage in and around the infarct zone, impairing ventricular remodeling."

Dr. Hazen thus believes MPO will have indications outside the ED. "Studies reported in abstract form but not yet in peer-reviewed publications show that MPO can predict risk in less-urgent-care situations," he says. An abstract presented at the American Heart Association's scientific sessions in 2004 by physicians at Emory University showed that MPO levels might flag subclinical coronary artery disease in those without obvious symptoms.

"And yet another recent study reported at AHA a few months ago showed elevated MPO levels were associated with the incidence of developing heart failure in apparently healthy middle-aged subjects on a community screen," Dr. Hazen adds.

How are cardiac biomarker panels likely to evolve? For detecting and managing

ACS, Dr. Morrow believes MPO is the “most promising candidate currently available” to add to a panel that includes troponin and BNP or pro-NT-BNP. “In the future,” he predicts, “there will likely be added inflammatory markers, markers of plaque instability, markers of thrombosis, and additional markers for hemodynamic stress.”

But based on past experience, he says, it is likely to be three to five years before sufficient evidence is seen to recommend the routine use of additional markers beyond troponin.

Clinicians will also face a learning curve in using newer markers, such as MPO, he notes. “And we need additional evidence as to how to respond to the newest markers that correlate with prognosis, such as BNP/NT-proBNP and MPO.”

Biosite’s Dr. Buechler agrees “it’s too early to tie MPO to available treatments and to look at changes in outcomes.” But “one thing is clear and somewhat intuitive,” he says. “If someone is suffering from an acute condition and that acute condition is life-threatening, the sooner you can identify the condition and use the available treatment, whatever that is, the better the patient is going to do.”

Dr. Hazen predicts more than one panel: one for situations that are urgent and one for risk screening in outpatient settings.

Family practice physician Bill Cayley Jr., MD, assistant professor at the University of Wisconsin Eau Claire Family Practice Residency Program, says as research into cardiobiomarkers continues, the question clinicians have to ask themselves is which tests are going to provide meaningful information to help them make clinical decisions.

“If a person is at moderate risk and a new test says the person is at a bit higher risk, that doesn’t change the picture much,” Dr. Cayley says. “Or if a person is low risk and a test confirms the person is low risk, that information doesn’t add anything—except expense.”

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