Reflections on the Evolution of Cardiac Biomarkers

Jack H. Ladenson1*

I was asked for this special issue to reflect on the area of laboratory tests for identifying cardiac injury. I will include my own experiences in this area, as well as some of others and a bit of personal perspective. I have published elements of this history previously (1–3) and freely admit to drawing on my prior publications.

Considerations for a blood protein biomarker to identify the death of any cell involve 3 major factors: sensitivity, which will be affected by the abundance and location of the protein in the cell; the timing of sampling, which is influenced by the mode of entry into blood and the half-life of elimination; and specificity for the cell of interest. A compounding factor in myocardial infarction (MI)2 (acute coronary syndrome) is that the acute event is caused by blockage of blood flow in a coronary artery or arteries that leads to hypoxia and cell death. This blockage delays protein markers from reaching the blood because they cannot readily diffuse into the blood; instead, they reach the blood via the lymphatics, which is somewhat akin to traveling on city streets rather than on an interstate.

Today, we have several discovery tools for identifying candidate cell injury biomarkers via gene expression (4) or proteomics (5) in a reasonably straightforward manner. It is noteworthy that cardiac biomarkers evolved without such tools, and appropriate biomarkers were found intuitively and empirically (Table 1), probably because the heart’s major function is contraction, which requires energy. The biomarkers used were all involved in energy production or control of contraction.

The first practical test used was serum glutamic-oxaloacetic transaminase (SGOT), now called “aspartate aminotransferase” (AST) (6). Practical use of this test in clinical laboratories required the development of robust, temperature-controlled spectrophotometers, such as the Gilford 300N developed by Sol Gilford, and a practical source of pure reagents, which was aided by the efforts of people such as Dan Broida at Sigma Chemical Company. As AST became more widely used, its nonspecificity for cardiac tissue (skeletal muscle damage and liver congestion) became appreciated, and other biomarkers were sought. In 1960, creatine kinase (CK) was demonstrated to be a possible biomarker of cardiac muscle damage, as were lactate dehydrogenase isoenzymes (2). Because CK activity is low in the liver, its use avoided the diagnostic problems of the increases in AST that can occur in liver damage and cardiac congestion. In the 1970s, electrophoresis for CK isoenzymes [MB isoenzyme of CK (CK-MB)] became commonly used. The work of Sidney Rosalki led to a modification of the Kornberg ATP assay for CK (1), which became the foundation of CK activity measurements. The work on standardizing CK activity measurements was extended by many workers, notably Gábor Szász, whose premature death was unfortunate for the world of science (2).

My own involvement with CK isoenzyme measurements started in the 1970s. My first experimental experience with these enzymes came via a biochemical teaching laboratory for medical students and involved David Bruns, a former editor of Clinical Chemistry and then a resident at Barnes Hospital. We had set up an example of enzyme reactions by using the assay for CK in patient serum. One of the students looking at the chart recorder (remember those?) said, “This thing isn’t working,” or something to that effect. It turned out that the sample came from a patient with metastatic cancer and had a highly prolonged lag phase in the enzymatic reaction for detecting CK (7).

In the 1980s, Washington University became involved in an academic–industrial research arrangement to develop monoclonal antibodies for use in the diagnostics and therapy of human disease. The original agreement was with a part of the Monsanto Company (now part of Pfizer) and Mallinckrodt (now part of Cvidien). Conflicts among the industrial partners were avoided by clearly delineating project areas along with proportional funding of core facilities, such as a monoclonal antibody fusion center and an immunoassay center. The agreements were modified after a few years, with Mallinckrodt ending their involvement and Monsanto broadening theirs into efforts more targeted at the discovery of novel drugs. The work that ultimately led to the development of a monoclonal anti-
body (Conan-MB) reactive only with the CK-MB isoenzyme, and quantitative tests for CK-MB (8) began as one of the Monsanto projects and involved my colleague Dave Dietzler, who was a very talented enzymologist (2). The work evolved rapidly after Sharon Porter and Hemant Vaidya joined our laboratory. It took a while to learn some of the nuances of developing monoclonal antibodies. The major factors we had to learn included avoiding the traditional screening assays—because they were prone to selecting for antibodies to epitopes on immobilized antigens, which were not present on soluble antigens (1, 2)—and the use of different strains of mice, because the commonly used BALB/c strain did not produce antibodies to CK-MB but the A/J strain did (2).

Conan-MB, a conformationally sensitive antibody, was first used as a capture antibody for CK-MB, with elution and measurement of CK activity following (2, 8). Later, it was paired with an antibody to the B subunit of CK-MB (referred to as Mr. Bill) to make a convenient, 2-site mass immunoassay. The assay was first made commercially available by the Dade Division of Baxter, Inc., in 1988; shortly thereafter, most other manufacturers made the assay available. Its arrival was timely because the demand for CK-MB analysis was climbing rapidly, probably because of the advent of new therapeutic interventions for MI, such as streptokinase [cleared by the US Food and Drug Administration (FDA) in 1982] and tissue plasminogen activator (FDA cleared in 1987). We were doing multiple runs of CK-MB activity by electrophoresis, and the laboratory people and the clinicians were getting frustrated with each other. It is interesting that the cardiac care unit at that time was using a CK-MB procedure based on batch absorption of serum with DEAE-coated glass beads (9). When the lot of beads the cardiac care unit was using had to be replaced, the new lots were not successful for the assay, and we started to supply the unit with Conan-MB—coated beads for use in a similar manner. This step ultimately led to consolidation of all CK-MB testing in the hospital laboratory.

The use of a facile and sensitive CK-MB mass assay indicated that CK-MB was not totally heart specific. When performing electrophoresis, we had to dilute samples to an activity of approximately 300 U/L to avoid overloaded the electrophoretic system and did not detect CK-MB very often in non-MI patients. With direct analysis of CK-MB irrespective of the total CK activity, it was quickly realized that CK-MB can come from skeletal muscle, and we and others then realized that that occurred in a variety of situations owing to skeletal muscle regeneration. This process was demonstrated elegantly in American professional football players by investigators in our institution (10). The CKB (creatine kinase, brain) gene is an embryonic gene, and its production is increased in regenerating skeletal muscle. Recognition of the occasional lack of specificity of CK-MB for MI accelerated the desire for a test more specific than CK-MB.

The name of the Conan-MB antibody came about as follows: We were working with several proteins and antibodies in the mid-1980s. A system of numbering was in use, which allowed tracing a variety of technical variables, such as the antigen preparation, fusion number, ELISA plate, and well number. When reviewing data concerning the various antibodies at laboratory meetings, we found it difficult to follow which was which. Vonnie Landt (née Maynard), my research associate, was asked to give the antibodies names to make it easier to follow the various experiments. Various names, such as Jack, Hem, Yvonne, and Fred (yes, that Fred), were used. The antibody that was identified to have the unique reactivity for CK-MB was named Conan. Vonnie was known to be a fan of the Conan movies starring Arnold Schwarzenegger.

In the 1980s, we began looking at cardiac troponin I (cTnI) and myosin light chain as possibly more specific cardiac biomarkers. Interest in cTnI was prompted by the work of Cummings et al. (1, 2); knowledge of the cTnI sequence by a colleague, Anne S. Murphy in the Pediatric Department (1, 2); and the reports of Katus et al. on the use of myosin light chain (11). There was no cardiac specificity with the myosin light chain assay developed by Emad Daoud in our laboratory, and the results attributed to myosin light chains later appeared to be due to cardiac troponin T (cTnT), which Katus’ group developed into a useful cardiac biomarker (12). The work with cTnI involving a postdoctoral fellow in the laboratory, Geza Bodor, was successful (13), and these antibodies are used in cTnI assays available through Dade International (now part of Siemens Healthcare). The arrangements with Dade International were part of a still continuing re-

**Table 1. History of use of biochemical markers of myocardial injury.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late 1950s</td>
<td>Aspartate aminotransferase (AST, SGOT)</td>
</tr>
<tr>
<td>1960s</td>
<td>Creatine kinase (CK, CPK*)</td>
</tr>
<tr>
<td>1970s</td>
<td>Creatine kinase isoenzyme (CK-MB activity)</td>
</tr>
<tr>
<td>1970s</td>
<td>Lactate dehydrogenase isoenzymes (ratio of LD1 to LD2)</td>
</tr>
<tr>
<td>Late 1980s</td>
<td>CK-MB mass concentration</td>
</tr>
<tr>
<td>Mid-1990s</td>
<td>cTnI, cTnT</td>
</tr>
</tbody>
</table>

* CPK, creatine phosphokinase; LD1, lactate dehydrogenase fraction 1; LD2, lactate dehydrogenase fraction 2.
search agreement that started in 1989. A major reason I agreed to this arrangement was the response of the then head of research of the Dade Division of Baxter, Susan Evans, to a problem that developed with the Dade Status CK-MB procedure. An inhibitor to human alkaline phosphatase used in the assay to avoid interference with the animal alkaline phosphatase detection system turned out to be unstable after some months. The research group at the company confirmed the assay problem, identified the cause, solved the problem, and partook in the publication of the problem and its solution (14). This type of intellectual honesty is somewhat rare, and it definitely influenced my decision regarding research arrangements. Around the same time, David Silva, a postdoctoral fellow, developed a 2-site immunoassay for myoglobin (1, 2), which has also been widely used.

The cTnI work after development of the assay was actually very challenging, because we thought we might have a test that performed better than the gold standard of CK-MB. Fortunately, I had excellent clinical researchers as collaborators, particularly Al Jaffe, Victor G. Dávila-Román, and a postdoctoral fellow in cardiology, Jesse Adams III. Al and Victor devised a means of detecting MI by serial echocardiograms, which kept us out of the logic loop of simply comparing 2 blood tests and trying to prove one is better by seeing who could shout louder.

With the ability to assess cardiac damage independently of the assay of CK-MB or cTnI, we embarked on a series of clinical studies to validate the specificity of the cTnI assay and to assess its value to detect myocardial injury in a variety of clinical situations (1-3). Since then, many others have confirmed the specificity of cTnI for heart damage.

The clinical studies with cTnI led to a few surprises. One was the time course after MI. The initial appearance of cTnI over time after an MI is similar to that of CK-MB, as is the initial decrease after the peak value. CK-MB returned to baseline after a few days, but TnI values still showed some increase for 7–10 days, although the half-life of cTnI is similar to that of CK-MB, a finding determined in collaboration with a colleague, Dana Abendschein. The current thought about this observation is that the initial increase in cTnI is attributable to the release of cytosolic TnI and that the extended increase is attributable to cTnI release from the myofibrils as the repair process proceeds in the heart.

This prolonged increase in cTnI turned out to be an advantage because it allowed cardiac troponin testing to replace lactate dehydrogenase isoenzymes for detecting cardiac damage in patients presenting a day or so after their MI. Lactate dehydrogenase isoenzyme testing was, in hindsight, a very poor test for patients without electrocardiographic evidence of MI (15). Another surprise was the prolonged increase in cardiac troponin over time in some patients with unstable angina, which was first reported for cTnT (16). Initially, some of us thought that that represented delayed presentation of patients with MI, but it is now clear that a subset of patients with a clinical diagnosis of unstable angina have low persistent increases in cardiac troponin and that this subset of patients has a prognosis similar to that of patients with non–Q-wave MI.

There was another interesting observation early in our clinical studies with cTnI. We had received deidentified samples from a group doing a charity race across the country. One of the samples from an approximately 70-year-old man had a very high cTnI value. Although we could not identify the individual, we were very concerned that heavy exercise might lead to extensive heart damage. As an aside, Al Jaffe, a cardiologist, was in a conundrum (probably a panic) about recommending against exercise. We put together a protocol to study ultramarathoners at high altitude but found no increases in cTnI in this group (17).

Since the development of rapid, robust assays for CK-MB, cardiac troponin, and myoglobin, there has been extensive work on additional biomarkers (18) and progressively more analytically sensitive cardiac troponin assays (19, 20). Although these assays are discussed elsewhere in this special issue of Clinical Chemistry, I hope there is also thought about the extreme difficulty of supporting such efforts in a routine clinical laboratory. Even today, the efforts of sustaining the long-term precision and accuracy for cardiac troponin across different lot numbers of materials and multiple instruments—even of the same model—takes considerable effort and skill to keep the system robust for the clinician.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: J.H. Ladenson, Siemens Healthcare Diagnostics.
Expert Testimony: None declared.
Other Remuneration: J.H. Ladenson, royalties from Siemens Healthcare Diagnostics. The author and Washington University may receive income on licensing of technology discussed in this report (troponin, myoglobin). The terms of the above arrangements are being managed by Washington University in accordance with its conflict-of-interest policies.

Clinical Chemistry 58:1 (2012) 23
References


