Cardiac Markers: From Enzymes to Proteins, Diagnosis to Prognosis, Laboratory to Bedside*

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ABSTRACT

For many years, serologic markers have been used to assist cardiologists in the diagnosis and management of patients with cardiovascular diseases. The use of laboratory markers has evolved and kept pace with the field of cardiology itself. The early markers involved testing for total enzyme activity such as aspartate aminotransferase, lactate dehydrogenase and creatine kinase. Shortly thereafter, the World Health Organization included serial enzyme markers as part of the triad for diagnosis of acute myocardial infarction (AMI). It was soon recognized that isoenzymes such as for CK-MB and LD-1 provided more specific organ specificity. The need for reporting rapid results led to the development of totally automated isoenzyme assays, which have evolved from immunoinhibition (INH) techniques to mass assays. The current emphasis for cardiac markers is use of protein markers such as cardiac troponin T (cTnT) and I (cTnI). These markers are more sensitive and specific than isoenzyme markers and enable risk stratification for non-AMI patients with unstable angina: patients with high troponin have a higher risk for AMI and cardiac death within the immediate future (4 to 6 weeks). Prospective management of cardiac patients requires more rapid testing and reporting of results. Point-of-care testing platforms on whole blood are now available for emergency testing at bedside.

Introduction

Cardiovascular disease continues to be the most prevalent cause of morbidity and mortality in the western world. Each year, hospital emergency departments see some 6 million Americans suffering from chest pain. Many of these patients have a diagnosis of acute coronary syndromes (ACS), i.e., the rupture of an unstable coronary artery plaque leading to unstable angina pectoris and acute myocardial infarction. Fortunately, cardiac injury from ACS can be reliably detected by the release of enzymes and proteins from the damaged myocardium to the circulation. The clinical sensitivity of these markers for diagnosis of AMI approaches 100 percent.

Enzymes as Markers of AMI

Most individuals in the field of laboratory medicine credit the work of Karmen, Wróblewski and LaDue for identifying, in 1954, the first biochemical marker for diagnosis of acute myocardial infarction (AMI) (figure 1).1,2 These investigators used paper chromatography to show that aspartate aminotransferase (AST, formerly known as glutamic oxaloa-
cetic transaminase) was increased by 2- to 20-fold over normal limits within 24 hours after AMI, and remained abnormal for 3 to 6 days later. The paper chromatography method was very laborious, requiring an 18-hour incubation step with the substrate solution. Karmen, however, developed a rapid spectrophotometric assay, by coupling oxalacetate produced in the AST-catalyzed reaction with malic dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH) to produce malate and NAD+. The reaction was monitored in a spectrophotometer by the decrease in light absorption at 340 nm. Although modified with the addition of cofactors, this original assay has essentially been in continuous use since this time.

Studies on the use of lactate dehydrogenase (LD) by these investigators were conducted and published within one year of their work on AST. Simultaneously, Wacker, et al., showed that LD was typically increased on the first day of infarction, peaked on day two or three, and returned to normal within 7 to 11 days. These investigators also suggested a diagnostic role for malic dehydrogenase for AMI patients, which did not ever become part of routine clinical practice.

The first diagnostic use of creatine kinase (CK, formerly creatine phosphokinase) was described in 1959 in patients with progressive muscular dystrophy. It was recognized in the following year that CK activity measurements could also be used in patients with AMI. In the early studies of Sorensen, the sensitivity of CK was 98 percent (52 of 53 patients) when blood was collected within 72 hours after the onset of disease. This investigator also suggested that AMI patients with high CK on the third day had a worse prognosis than those with activities within the reference range. The role of CK for myocardial infarct sizing may have been first established by these observations. Modern spectrophotometric measurements for total CK are credited to methods developed by Oliver, who described the coupling of ATP from the reverse CK-catalyzed reaction to hexokinase and glucose-6-phosphate dehydrogenase, and Rosalki, who identified the importance of thio compounds such as cysteine to prevent loss of enzyme activity after blood collection.

The importance of enzyme measurements for the diagnosis of AMI was recognized by the World Health Organization when it was included as part of the triad for diagnosis of AMI, along with the clinical history of chest pain at presentation and specific electrocardiographic changes. With the development and widespread use of other protein markers that
are not themselves enzymes, the laboratory portion of this 1979 AMI definition is in need of modification.

Isoenzymes and Isoforms

Although measurement of total enzyme activity had good sensitivity for diagnosis of AMI, it was recognized very early that the specificity of these markers was less than ideal. Patients with disorders of the liver, biliary tract, kidneys and skeletal muscles produced falsely positive results. The measurement of specific isoenzymes was proposed by Wroblewski as a means to increase the specificity of cardiac enzyme markers.12 Early methods for isoenzyme analysis for CK and LD were zone electrophoresis.12,13 Bands are visualized by incubating gels with standard enzyme reagents and quantitated by densitometry. In 1974, Mercer described an alternative technique for measuring CK and LD isoenzymes using an anion-exchange column chromatography.14 Serum samples were loaded onto a column packed with DEAE Sephadex A-50, eluted into separate isoenzyme fractions with increasing concentrations of a salt solution and assayed for residual CK and LD activity.15 While electrophoresis and an automated chromatographic assays were very popular for CK isoenzymes for many years, they have largely been replaced by CK-MB mass assays.

The next generation of isoenzyme assays involved the use of antibodies raised against these enzymes. In the immunoinhibition technique, developed in the late 1970s, antibodies were used to inhibit non-cardiac specific isoenzymes selectively, leaving the targeted cardiac-specific isoenzymes (CK-MB and LD-1) active for direct enzyme analysis. For CK-MB, antibodies to the CK-M subunit were added to inhibit CK-MM and half of the CK-MB activity. The CK-MB activity was calculated as the residual B-subunit activity times two.16 The immunoinhibition assay for CK-MB was not specific, as the presence of CK-BB and macro CK forms produced positive interferences.17 Nevertheless, the INH assay can be automated onto general chemistry analyzers and is still widely used today. For LD-1, antibodies to the M-subunit were added to tag and precipitate non-cardiac LD isoenzymes 2-5.18 Chemical inhibition assays for LD-1, such as with guanidine thiocyanate or sodium perchlorate, have also been developed. These assays do not involve use of specific antibodies and correlate strongly to immunoinhibition assays.19

The first mass assays for CK-MB were developed in the mid-1980s20,21 These assays measure the concentration of the protein rather than its enzyme activity. They are more sensitive than activity measurements, and more specific than immunoinhibition. In 1986, Vaidya, et al, developed the first monoclonal antibody to CK-MB.22 This antibody is now incorporated into nearly all commercial assays for CK-MB. The initial mass assays for CK-MB were manual, time-consuming and impractical for stat use. Today, these assays are part of automated immunoassay instrumentation, with a typical on-instrument turnaround time of 10 to 20 minutes.

In addition to CK isoenzymes, there has been interest in measurement of CK isofoms (or subforms), which are subsets of major CK isoenzymes.23 There are three CK-MM and two CK-MB isoforms that exist in serum, produced as the result of postsynthetic degradation by serum carboxypeptidase of single isoforms found in muscle tissues. The relationship of the two CK-MB isoforms provide diagnostic information that is earlier than measurement of total isoenzyme activity alone and may be useful for early triage of chest pain patients.24 Only high-voltage electrophoresis assays are currently available for CK-MB isoforms.25

Protein Markers

Protein markers for AMI diagnosis were first proposed in the late 1970s with the development of assays for myoglobin.26 Due to the small size of this heme protein, it was recognized very early that myoglobin would provide early diagnostic information for patients who present to the emergency department with chest pain. Unfortunately, the original assays were radioimmunoassays and could not therefore be used for stat analysis. Automated
non-isotopic immunoassays for myoglobin, which enable testing to be performed with a rapid turnaround time, were developed in the late 1980s. Today, myoglobin is used to support hospitals that have a rapid AMI rule-out protocols. These emergency department (ED) "chest pain" centers combine frequent testing of cardiac markers with provocative testing such as a stress treadmill to triage and risk stratify patients within 9 to 12 hours after ED presentation. Myoglobin enables rule out of AMI within 6 hours after ED admission, about 3 hours earlier than CK-MB.

The newest protein markers are for cardiac troponin T (cTnT) and I (cTnI). These markers are highly specific for myocardial damage because the cardiac isotypes are not found in any other adult tissues, including skeletal muscles. Therefore, assays can be developed such that elevations of these markers are indicative of only cardiac injury. Clinical studies have shown that the cardiac troponins are equivalent to CK-MB for diagnosis of AMI. More significantly, however, assays for cTnT and cTnI have a role in predicting future coronary risks in patients with unstable angina (UA). Clinical studies have shown that UA patients with high cTnT or cTnI have about a 5-fold higher incidence of AMI or cardiac-related death within 4 to 6 weeks than a cohort of UA patients without increased troponin concentrations in blood. These studies suggest that cTnT and cTnI are detecting ischemic injury or microinfarcts that are not discernable by other less sensitive cardiac markers such as CK-MB or myoglobin, or techniques such as the electrocardiogram. Thus there may be a paradigm shift towards the use of cardiac troponin for prospective management—and not just for diagnosis—of coronary heart disease.

Revascularization Therapy and Cardiac Markers

The "closed artery theory" of thrombosis as the major cause of acute myocardial infarction was first proposed in 1912. Nevertheless, the notion that occluded coronary arteries should be aggressively recanalized as soon as possible has been universally accepted by all cardiologists only within the last decade. Today, ED physicians and cardiologists have a variety of therapeutic options for AMI patients, including intravenous thrombolytic therapy such as with streptokinase, urokinase and tissue plasminogen activator, and surgical procedures such as coronary angioplasty, directional arthrectomy, stent placements and coronary artery bypass graft surgery. An active area of research is the measurement of cardiac markers to determine the success of revascularization therapy to open arteries. Myoglobin appears to be the best but more work is needed in this area.

Point-of-Care (POC) Testing

The increasing emphasis of cardiac markers on the acute management of cardiac patients requires results of tests to be available in real time. Laboratory-based assays for cardiac markers require a minimum of 30 to 60 minutes for delivery of the sample, centrifugation to obtain serum or plasma, analysis and reporting. Point-of-care testing is becoming increasingly important for hospitals that cannot consistently deliver an adequate turnaround time for clinical needs. POC testing devices based on whole blood analysis are now available for myoglobin, CK-MB, cTnT and cTnI and offer a low-cost alternative. Qualitative tests have a cutoff concentration that designates the presence of significant cardiac injury indicative of AMI. With serial blood collections, quantitative tests enable tracking of the typical rise and fall of the markers after AMI. Marker concentrations can also be used to estimate the size of the AMI. The National Academy of Clinical Biochemistry has recommended use of a panel of these markers, to include myoglobin or CK-MB with cardiac troponin (T or I) for optimum use of these tests.

References