Enzymatic Diagnosis of Acute Myocardial Infarction

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The diagnosis of acute myocardial infarction for some time has been based on the World Health Organization's "two out of three" criteria, namely: angina, electrocardiographic changes, and elevated plasma enzymes. This was devised in 1959 before clinical application of plasma isoenzymes. It was soon realized that Q-waves on the ECG, while very specific, are not very sensitive. The combination of chest pain and nonspecific ECG changes, namely: alterations in the ST-T segment, does not differentiate angina from infarction. Similarly, elevated plasma activity of LDH, SGOT, or CK with nonspecific ECG changes or chest pain could incorrectly include many patients with chest pain without cardiac necrosis, or even cardiac disease, particularly if associated with minor trauma such as from intramuscular injections. The diagnostic application of isoenzymes, the widespread availability of the coronary care unit (CCU), and the implementation of interventions early during the course of acute myocardial infarction (AMI) stimulated the need for an earlier and more precise diagnosis. At the present time, it is estimated that only about 25-30 percent of patients admitted to the CCU are subsequently proved to have AMI. The need to be cost-effective and to appropriately allocate critical care beds requires an early diagnosis to determine who can be appropriately transferred and when. The recent widespread use of early thrombolytic therapy provided further impetus since, when successful, the rapid washout enhances release of cardiac enzymes and accelerates the evolution of ECG manifestations.

Sampling for Creatine Kinase

Routine Infarction

It is now generally recognized that serial analysis of plasma MB CK activity provides the most sensitive, specific, and cost-effective means of diagnosing AMI. The tradition of assaying three enzymes, LDH, CK and SGOT (AST), daily or twice daily is to be avoided. It is only necessary to analyze total CK and MB CK routinely, and it should be performed frequently (such as every four to six hours). Plasma MB CK activity as assessed by conventional techniques is significantly elevated within four to six hours of onset of symptoms of infarction, and maximum levels are reached between ten and 36 hours with return to normal levels after 48 to 72 hours. In patients with minimal cardiac injury, such as occurs in non-Q-wave infarction, MB CK peaks on an average of 15 hours. In contrast, after Q-wave infarction, it peaks at around 28 hours. It is recommended that samples be analyzed on admission and every four to six hours for the initial 24 hours. The diagnosis is usually evident within eight to 12 hours, and frequent further sampling is unnecessary. Frequent sampling is recommended, as about 65 to 75 percent of patients will not have infarction and thus can be transferred to the ward or home within eight to 12 hours. Sometimes it might be judicious to sample every 30 to 60 minutes, as a diagnosis can often be made within one to two hours. If for prognostic or other purposes one needs to assess peak plasma enzyme activity or infarct size, serial analysis every six hours is necessary for at least 48 hours. In patients with non-Q-wave infarction, because of their high propensity for early reinfarction, sampling should be continued every 12 hours throughout the hospital stay, as it should be in any patient considered at high risk of reinfarction. To be cost-effective, one may initiate sampling only if the patient develops chest pain, ECG changes, or other features suggestive of infarction. This approach seems reasonable since in the recent Diltiazem Reinfarction Study, consisting of 576 patients with non-Q-wave infarction in whom 43 percent had chest pain, the accumulated incidence of patients with elevated MB CK activity was only 12 percent. In patients who develop recurring pain or other features suggestive of reinfarction, samples should be collected every four to six hours for 12 to 24 hours.

Infarction with Thrombolytic Therapy

In patients who receive thrombolytic therapy or angioplasty (within four to six hours), MB CK should be assessed hourly for the first four to six hours, followed by every four hours for 36 hours, and sampling reinitiated if chest pain or other features occur to

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CHEST / 90 / 1 / JANUARY, 1986 / Supplement 35
suggest reinfarction. Due to rapid washout, as occurs with successful thrombolysis, a significant elevation in MB CK is detected within 30 to 60 minutes of reperfusion, and plasma activity reaches a maximum within ten to 15 hours. Early diagnosis may be important for subsequent stratification to further thrombolytic therapy or to angioplasty or surgery. Patients undergoing selective coronary angioplasty in whom it is suspected that infarction may have occurred should undergo a sampling regimen similar to that following thrombolytic therapy.

Infarction after Surgery

Myocardial infarction after noncardiac surgery is also reliably determined from serial analysis of plasma MB CK every four to six hours. There is marked elevation of other enzymes due to tissue trauma, but MB CK is highly specific. In the setting, however, of cardiac surgery, MB CK, like other cardiac enzymes, is almost always elevated due to manipulation and involvement of the myocardium and, thus, is not a reliable diagnostic index and should be diagnosed by radionuclide techniques, as indicated in the article by Dr. Willerson. Nevertheless, plasma MB CK elevated several-fold in the absence of Q-waves is highly suggestive of perioperative infarction, although as a sole criterion it lacks specificity.

Diagnosis in Patients Admitted Late

In patients admitted within 48 to 72 hours of onset of symptoms, plasma MB CK is extremely sensitive as well as specific; however, in patients admitted after 72 hours, particularly when associated with minimal myocardial damage, MB CK may have returned to normal levels. Thus, in this situation, it is more appropriate to analyze for LDH-1 activity which peaks between 48 and 72 hours and remains elevated for ten to 14 days. It is estimated that in less than 5 percent of cases is it necessary to analyze for LDH activity. Total CK, although a highly sensitive index of infarction, is less specific and may be elevated after a variety of conditions, such as intramuscular injection, trauma, cardiac catheterization, surgery, cerebrovascular accidents, stroke, or thyroid disease. Electrical cardioversion causes significant elevation of plasma total CK activity, but unless repeated several times, does not elevate MB CK. It is also important that MB CK be analyzed even if total CK activity is within the normal range. The upper limit of normal is such that patients with low baseline levels may increase their total and MB CK activity two- to three-fold while total CK activity remains in the normal range.

Detection of Extension or Early Reinfarction

Diagnosis of early reinfarction was difficult until the advent of quantitative assays for MB CK. Plasma LDH isoenzyme activity does not reach a peak until 48 to 72 hours and remains elevated for ten to 14 days, and with the lack of a precise quantitative assay for LDH-1 activity, it was difficult to detect a secondary elevation that could be interpreted reliably as new necrosis. This is further compounded by the fact that a source of LDH activity is the inflammatory infiltrate. The advent of rapid, quantitative, and sensitive assays for MB CK and the observation that MB CK peaks on the average of 24 hours, paved the way for more precise diagnosis. Nevertheless, it is still difficult to interpret from MB CK analysis whether extension occurs in the first 24 hours; however, after MB CK has reached a peak, a secondary elevation on the downslope can be detected, but only with a quantitative assay. Detection of early reinfarction in the first 24 hours is more feasible in patients who undergo successful thrombolysis, since MB CK activity usually peaks within the first ten to 15 hours. A secondary elevation of MB CK activity 36 to 48 hours after onset of symptoms is relatively easy to detect and provides a sensitive and specific diagnosis of early reinfarction. In the latter situation, we define reinfarction, based on the quantitative glass bead assay, as an increase of 50 percent or more of the plasma MB CK activity above the preceding baseline (mean of two preceding samples) in at least two samples separated by a minimum of four hours within a 24-hour interval with an absolute value of ≥14 IU/L in at least one sample. If the MB CK activity is on the downslope from the antecedent infarction, then a 25 percent increase is considered diagnostic; however, this is always less reliable than a secondary elevation after return of activity to baseline. These criteria were used in three large clinical trials: Nifedipine in Acute Myocardial Infarction Study (NAMIS), Multicenter Investigation of the Limitation of Infarct Size (MILIS), and Diltiazem Reinfarction Study (DRS).

Storage, Assays and Enzyme Criteria for MB CK

Samples for CK analysis should be collected in EGTA (5 mmol/L) and mercaptoethanol (16 mmol/L). Samples should be frozen; however, activity can be maintained in a refrigerator for at least 24 hours. Analysis is generally performed by the qualitative analysis using electrophoresis; however, the quantitative techniques are preferred. The electrophoretic techniques provide adequate sensitivity and specificity most of the time for diagnosis of myocardial infarction, but cannot be so easily interpreted with respect to the
diagnosis of early reinfarction and does not lend itself to the same serial analysis as the quantitative assays. It is recommended that one use one of the quantitative assays—either the glass bead assay or radioimmunoassay. In our own laboratory, the upper limit of normal using the glass bead assay is 13 IU/L for MB CK, for the radioimmunoassay 40 μg/L, and for total plasma CK activity 120 IU/L. These values, however, will vary among laboratories and must be established for each laboratory. Normal myocardium contains on the average about 15 percent MB CK; the remainder is MM CK. Other sources of MB CK, such as certain skeletal muscles or the small intestine, contain only trace amounts in the range of 1-2 percent. Massive skeletal muscle injury is associated with significant release of MB CK, although it seldom forms more than 1-2 percent of total activity and, thus, in diagnosing myocardial infarction, it is generally stated that MB CK activity must exceed 5 percent of total activity.

At the time of peak activity following myocardial injury, generally MB CK level comprises 10-15 percent of total CK activity. The following guidelines are suggested as enzymatic criteria for the diagnosis of myocardial infarction: 1) if serial samples are available, a serial elevation followed by a decrease in plasma MB CK to baseline levels with a change of 25 percent of more between any two values; 2) an increase in plasma MB CK activity of 50 percent or more between two samples separated by at least four hours and not more than 12 hours; 3) the diagnosis is made preferably on not less than two samples in a 24-hour period separated by at least four hours; 4) if only a single sample is present, diagnosis must be made on the basis of an elevation above normal by at least two-fold; 5) in patients admitted beyond 72 hours from onset of infarction, LDH isoenzyme analysis is preferred since MB CK may have returned to normal.

LDH ACTIVITY

Plasma LDH activity reaches significant levels much later than MB CK and peaks at about 48 to 72 hours, remains elevated for ten to 14 days and, thus, in the case of late admission (>48 to 72 hours) is a more appropriate diagnostic marker. It is recommended that in the CCU, LDH activity level is required in less than 5 percent of patients. It is, thus, recommended that patients admitted within 48 hours undergo sampling for MB CK only, but later than that, samples should be drawn on admission for LDH as well as CK, and if MB CK is not elevated, then analysis be performed for LDH-1 isoenzyme. It is recommended that analysis be for the isoenzyme LDH-1 since it is fairly specific for the myocardium, whereas skeletal muscle and liver are rich in LDH-4 and LDH-5 rendering total LDH activity far less specific. One of the more popular assays for measuring LDH activity is the so-called HBD which uses 2-oxybuterate for substrate instead of pyruvate. Two-oxybuterate is preferentially reduced by LDH-1 and LDH-2 which include the more specific isoenzymes of cardiac tissue. However, while the affinity is much less, it does serve as a substrate for other isoenzymes, and high levels of LDH-4 or LDH-5 would show up as a significant elevation in HBD and, thus, may be incorrectly interpreted as being LDH-1. It is recommended that LDH isoenzymes be analyzed by either electrophoresis or one of the immunoinhibition techniques rather than by the HBD method. Sampling for LDH activity due to its long half-life can be much less frequent than for CK with every 12 to 24 hours being quite adequate. For some time it was conventional to also assay plasma SGOT (AST); however, since it is present in several organs, including the liver, skeletal muscle, gastrointestinal tract, and lungs, it is very nonspecific, and if CK or LDH analysis is available, it represents an unnecessary cost. Serum AST is released within eight to 12 hours from onset of symptoms, reaches a peak in 48 to 72 hours, returning to normal within five to six days.

NEW TRENDS IN ENZYME ASSESSMENT

CK Subforms and Reperfusion

To date, there is no precise noninvasive means of detecting whether thrombolysis has been achieved even though manifestations such as relief of pain or rapid evolution of electrocardiographic changes are suggestive. Similarly, early peaking of plasma total CK or MB CK is suggestive but lacks necessary sensitivity and specificity. In 1982, we showed that MM CK upon release into the circulation undergoes hydrolysis by carboxypeptidase-B which cleaves the lysine from the M subunit, resulting in a more negatively charged molecule, with the result that on high resolution agarose or polyacrylamide electrophoresis, available to any hospital laboratory, MM CK is separated into three forms: MM-3, unmodified form newly released from tissue; MM-2, with just one subunit modified; and MM-1, the most negatively charged with both subunits modified. Two subunits are also recognized for MB CK. Under baseline conditions, MM-1 predominates (80 percent), but the profile changes rapidly after onset of infarction to MM-3 the predominant form. This may occur despite only minimal elevation in total CK activity, affording a much earlier diagnosis of infarction than with MB CK or total CK. Secondly, by analysis of the temporal profile, one can date the onset of infarction to within hours by detecting an increase in the tissue form, MM-3. Analysis of MB CK subforms and the MM CK subforms are now undergoing considerable interest as potential noninvasive markers for detecting reperfusion following thrombolytic therapy. Rapid rise in the tissue form,
MM-3, with rapid disappearance within 16 to 18 hours offers a means to determine whether reperfusion is successful. The subform MM-3 is also a very sensitive marker for detecting early extension during the first 24 hours. The use of the subforms at present is only experimental, but the clinical application is limited at the moment only by the development of more quantitative and convenient assays, which is likely to occur in the very near future. Another experimental technique now undergoing clinical trials for the diagnosis and assessment of myocardial infarction is that of myocardial uptake of FAb fragments to myosin light chain, described in more detail in the section on radionuclide assessment.

BIBLIOGRAPHY


