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Biochemical Diagnosis of Myocardial Infarction

Craig C. Foreback, PhD*

A rapid, sensitive, and specific marker for the diagnosis of acute myocardial infarction (MI) and the assessment of reperfusion following thrombolytic therapy has been sought by research workers for years. Creatine kinase-MB (CK-MB) is the best biochemical marker currently available to the cardiologist and the emergency room physician for the assessment of patients presenting with symptoms of acute MI. CK-MB is best measured using immunoassay techniques at 3- to 4-hour time intervals during the first 12 hours after onset of symptoms. Other currently available markers include lactate dehydrogenase and its isoenzymes and myoglobin. Future developments include assays for troponin, reported to be a true cardiac-specific marker, and myosin light chains which may have value in noninvasive infarct sizing. (Henry Ford Hosp Med J 1991;39:159-64)

Acute myocardial infarction (MI) is one of the consequences of severe atherosclerosis resulting in ischemia of the cardiac muscle. In its most severe form the blood supply may be completely blocked, leading to intense chest pain that is often described as crushing or tightness. Other symptoms include nausea and vomiting, profuse sweating, and pain radiating to the arms, wrists, or jaw. Blood pressure and heart rhythm may be normal or the patient may be in circulatory shock. Suspected acute MI results in over 4 million hospital admissions each year in the United States alone. Approximately 1.3 million cases of acute MI are diagnosed each year, resulting in 600,000 deaths. With the average cost of a cardiac care unit (CCU) bed approaching \$1,000 per day and considering that CCU bed space is always at a premium, there is intense interest in appropriate diagnosis and treatment of acute MI.

Clinical suspicion of acute MI affects both short- and long-term management decisions. Attempts to salvage myocardium through reperfusion should be made in the first 4 to 6 hours from the onset of infarction. Thus, patients presenting to the emergency room present a diagnostic dilemma.

With many patients, history and symptoms clearly indicate that a MI has occurred. The ECG often firmly establishes the diagnosis. The ECG can show changes characteristic of acute MI, such as changes in the ST segment and appearance of Q waves and T waves, provided the ECG has been normal previously. Because these specific changes can sometimes be obscured by previous cardiac events or may not appear at all (Q waves do not appear in subendocardial infarctions), the diagnostic sensitivity of the ECG can be as low as 62%, although its diagnostic specificity is nearly 100%. Nearly 50% of patients with an acute MI present to emergency departments with nondiagnostic ECGs. About 25% of all acute MIs may be clinically silent, associated with atypical symptoms or no symptoms. Many patients with silent infarcts are diabetic and their lack of pain is attributed to

the autonomic neuropathy associated with a long history of diabetes. In elderly patients acute MI often presents with sudden shortness of breath, confusion, fainting, or even a stroke. In those cases where history is atypical and/or the ECG pattern is equivocal, the clinician must rely on serum enzymes and other biochemical tests.

Laboratory Testing in Myocardial Infarction

Historically, creatine kinase (CK), aspartate transaminase (AST) (glutamic-oxaloacetic transaminase), and lactate dehydrogenase (LD) were the key enzymes used in the investigation of MI. Typically all three of these enzymes would be measured at periodic intervals for up to three days following a suspected acute MI. With the availability of isoenzyme determinations, requests for cardiac enzymes now include CK and LD isoenzymes. AST is no longer recommended as part of a cardiac profile; indeed, most experts agree that the isoenzymes of CK and in certain cases LD should be determined following acute MI. It is clear that CK-MB has become the most important serum enzyme test used in the diagnosis of acute MI. Other important markers which are utilized or under study for the diagnosis of acute MI include troponin, myosin light chains, and myoglobin.

Troponin

Although CK-MB is the best currently available marker for acute MI, it does have limitations. CK-MB can be elevated after

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skeletal muscle injury as the skeletal muscle regenerates. Another shortcoming of CK-MB measurement is that cardiac CK-MB returns to normal within 24 hours after the event. Thus patients admitted to the hospital two or three days postinfarction will likely have normal CK-MB.

A better marker for acute MI would be present only in cardiac tissue, would be released within a few hours of the event, and would remain elevated for days rather than hours. Recent studies have shown that certain elements of the troponin complex may meet these desired characteristics.

Troponin is a complex of three nonidentical polypeptides: troponin C (TnC) which binds calcium, troponin T (TnT) which binds tropomyosin, and troponin I (TnI) which inhibits ATPase activity. Each of the different subunits of troponin occurs in various isoforms according to the muscle type of its origin. TnC is known to exist in cardiac and fast-twitch (short duration action potential or fast channel activation of contractile machinery in skeletal muscle) skeletal isoforms. The slow-twitch (long duration action potential or slow channel activation) isoform appears to be identical to cardiac TnC and has been found in fibroblasts. Thus TnC is not specific for detection of myocardial injury.

TnI and TnT are known to exist in cardiac as well as slow-twitch and fast-twitch skeletal muscle forms. Their rapid release from ischemic muscle coupled with their cardiospecificity make them excellent candidates for laboratory markers of acute MI.

Cummins and associates (1) described the first radioimmunoassay (RIA) for cardiac TnI measurement. Thirty-two patients known to have had acute MI exhibited elevated TnI values 4 to 6 hours after the onset of chest pain. The early release pattern of cardiac TnI following acute MI was similar to the release of CK-MB; however, TnI remained elevated for more than four days. In another report, Cummins et al (2) carried out a comparative study of total CK, CK-MB, myoglobin, tropomyosin, and cardiac TnI. Only the mean cardiac TnI remained normal in marathon runners following a race.

Bodor et al (3) have described monoclonal antibodies for cardiac TnI. Preliminary results of these assays using these antibodies have been published.

Katus et al (4) have recently evaluated an enzyme-linked immunoadsorbent assay for the other cardiac-specific troponin, TnT. The method is based on a pair of monoclonal/polyclonal antibodies. Elevated TnT levels were found in acute MI patients as early as 3 hours postinfarction. Values remained elevated for up to three weeks.

These studies strongly suggest that troponin I or troponin T measurements have promise as biochemical markers for the diagnosis of acute MI. What we need now is a commercially available, rapid, monoclonal-based assay. At least one manufacturer claims to be developing such tests. This would allow testing on a large number of patients, leading to the description of the release characteristics of the troponin molecule following myocardial ischemia and reperfusion of myocardium.

Myosin light chains

Myofibrillar proteins such as myosin are major constituents of muscle. In 1978 Trahern and coworkers (5) reported on the

diagnosis of acute MI by immunological detection of myosin light chains in serum.

Myosin light chains are located at each amino-terminal part of the myosin heavy chain molecule. The amino terminal ends form the globular myosin heads which interact reversibly with actin during contraction. During acute MI, myosin light chains are released continuously within 3 hours after the onset of pain and for at least two and in some cases more than three weeks.

The kinetics of myosin light chain release are clearly different from cytosolic molecules like myoglobin or CK. Serum concentration of myosin light chains is not affected by reperfusion of the infarct zone. Thus cardiac myosin light chains may be particularly useful for the estimation of infarct size in either the non-reperfused or the reperfused patient.

A cardiac-specific light chain assay may not be achievable, however. There is evidence that the "cardiac" myosin light chain-1 isoform may also be expressed in slow-twitch skeletal muscle. Differentiation of cardiac and skeletal muscle damage may not be possible even with a highly specific anticardiac myosin light chain antibody.

Cardiac myosin light chain measurements allow sensitive detection of myocardial cell damage over an extended period of time and can be used as an indicator of infarct size. However, the lack of cardiac specificity of these assays may reduce its effectiveness as a biochemical marker in acute MI. The role of myosin light chain assays remains to be elucidated.

Myoglobin

Of all the markers of tissue necrosis discussed herein, myoglobin is one of the earliest to appear in the circulation. It also normalizes quickly after acute MI. Thus, myoglobin can be useful for detection of infarct extension or reinfarction. Recently developed methods for myoglobin are reliable and reasonably rapid.

In the absence of fibrinolytic therapy, serum myoglobin reaches levels above the upper limit in 3 to 4 hours. Maximum concentrations are reached in 8 to 12 hours. Peak values are followed by a rapid decrease in concentration, returning to normal within 24 to 36 hours. As with other tissue markers, myoglobin release is accelerated by fibrinolytic therapy.

While myoglobin appears to be useful during the early phases of an acute MI, it has some drawbacks, particularly its lack of organ specificity as myoglobin is found in both cardiac and skeletal muscle. Myoglobin concentrations increase after intensive exercise in nearly all individuals. Impaired renal function is also a factor responsible for an increase in myoglobin levels.

Because of its nonspecificity and because myoglobin levels are so transient, the use of myoglobin as a marker of MI is limited. It may, however, be extremely useful for the early diagnosis of an acute MI in patients who are hospitalized shortly after the onset of chest pain where trauma is not a factor. Current RIA methods for myoglobin determinations still restrict timely availability of data to the clinician. New methods that employ latex agglutination are currently under study. Such methods will make possible automated techniques that will provide results in a few minutes.

Creatine Kinase, Lactate Dehydrogenase, and Their Isoenzymes

Creatine kinase

CK is a dimeric enzyme composed of two subunits and has a molecular weight of 82,000. The subunits are the result of two distinct structural genes and are labeled M (muscle) and B (brain). These can then form three different isoenzymes: BB (CK-1), MB (CK-2), and MM (CK-3). The tissue distribution of the various isoenzymes is shown in Table 1. Other forms of CK have been reported including macro-CK, mitochondrial CK, and CK-MM and MB variants. Macro-CK is actually CK-1 associated with IgG. Mitochondrial CK is not related to either the M or the B subunit and is produced by a separate gene. A macro-CK type 2 has been reported and is a polymer of mitochondrial CK having a molecular weight of > 300,000 Daltons. At least three MM and two MB variants (isoforms) have been reported in serum following acute MI (6,7). Once released into the bloodstream, CK loses terminal amino acids by the action of carboxypeptidase-N present normally in the circulation yielding the different isoforms. This metabolism continues resulting in fractions which do not exhibit enzymatic activity. These isoforms, especially the MB isoforms, reportedly are useful in the early diagnosis of acute MI (8,9). The MM-3 isoform is said to be useful in assessing reperfusion following thrombolysis (10).

Separation and quantitation of CK isoenzymes

Over the last two decades three viable approaches have been described for the separation and quantitation of CK isoenzymes: electrophoresis, ion exchange chromatography, and immunological procedures.

Electrophoresis was the first method to achieve popularity in clinical settings and continues to be a popular procedure accounting for 40% of current practice. Separation on agarose or cellulose acetate has been used to separate the CK isoenzymes satisfactorily. The bands can be visualized by incubating the support with a concentrated solution of CK assay mixture following separation. The NADPH formed in the reaction is quantitated by fluorescent densitometry, which can detect activities as low as 2 to 5 U/L. Electrophoresis can detect many abnormal CK bands. Using isoelectric focusing techniques, CK can be separated into at least three different MM isoforms and two MB isoforms. The isoforms of CK are reported to be useful in three areas related to acute MI: early diagnosis, dating of onset, and determination of reperfusion.

Ion exchange chromatography can also separate CK isoenzymes. Ion exchange chromatography was a popular technique in the mid to late 1970s and is employed today in many laboratories combined with immunoinhibition on the Dupont aca clinical analyzer. DEAE-Sephadex A50, usually in the form of a mini-column holding about 60 mg of gel, is most commonly used and is available in commercial prepacked columns. CK isoenzymes are absorbed onto the gel. The gel is then washed and the CK-3 is eluted with 50 mmol/L, pH 8.0 Tris buffer containing 100 mmol/L NaCl. CK-MB is eluted next with the same Tris buffer containing 200 mmol/L NaCl. Finally CK-BB is eluted with Tris buffer, 50 mmol/L, pH 7.0 containing 500

Table 1
Tissue Localization of CK Isoenzymes

Isoenzyme	Tissue
BB	Brain
	Smooth muscle
	Thyroid
	Lung
	Prostate
MB	Cardiac muscle
	Tongue
	Diaphragm
	Skeletal muscle (trace amounts)
MM	Skeletal muscle
	Cardiac muscle

CK = creatine kinase.

mmol/L NaCl. Since the elution process dilutes the CK isoenzymes, it is important to use small volumes of elution buffer and/or the eluate as part of the solvent used for reconstitution of the CK reagents. The method is capable of detecting 1 to 5 U/L. It is relatively simple to perform but in the presence of high CK-MM there is a greater risk of carryover into the CK-MB fraction.

Immunological procedures such as immunoinhibition, immunoinhibition combined with immunoprecipitation, and enzyme immunoassay (EIA) are becoming increasingly popular. EIAs measure enzyme mass and are reported to be more sensitive than activity measurements. Immunological methods for CK isoenzymes usually require specific antisera against the M or the B subunits; however, immunoassays specific for MM isoforms have been reported. Many of the newer methods for CK-MB employ monoclonal antibodies against the entire MB molecule. Rabbit antisera can be used to precipitate specific isoenzymes out of solution in immunoprecipitation. They can also be used to competitively bind specific isoenzymes in immunoassay techniques. Immunoinhibition methods are more effective when goat antisera are employed as specific subunit inhibitors. Immunoinhibition and immunoprecipitation techniques measure residual enzyme activity while immunoassays such as RIA or EIA measure enzyme mass. Immunoassays can determine enzyme mass even when catalytic activity is lost. EIA or fluorescent immunoassay using a "sandwich" technique is currently the most popular of the immunoassay approaches. These immunoassays reportedly have higher analytical sensitivity than do methods that measure catalytic activities; however, it cannot be assumed that the diagnostic sensitivity is also better. Reports have shown diagnostic efficiency to be somewhat better than conventional methods of quantitation. There are several automated immunoassays now available such that labor requirements are less than the traditional methods, thereby offsetting the higher reagent costs.

Controversy exists primarily around which separation and quantitation technique is preferred. As previously mentioned, electrophoretic techniques for the identification and quantitation of cardiac isoenzymes are still widely used. Electrophoresis provides useful clinical data for the confirmation of acute MI, and recent advances in instrumentation, although expensive, have led to complete automation, allowing high throughput

with minimal labor. Electrophoretic methods generally are performed only during routine working hours in relatively large batches due to their complexity.

The immunoprecipitation and/or immunoinhibition techniques are widely used for LD and CK isoenzymes and their clinical utility has been demonstrated. In particular, the Roche Isomune procedures have been validated in a clinical setting. Immunoinhibition CK-MB assays without immunoprecipitation can potentially have a higher incidence of false-positive results because they are subject to interference from the CK-BB isoenzyme. While the presence of CK-BB in the general population is observed infrequently in those suspected of having acute MI, it can make a significant contribution to the rate of false-positives when using non-MB specific methods.

EIAs have become realistic alternatives for identifying and quantitating CK-MB. They are specific and analytically more sensitive than previously described techniques. Currently available instrumentation has completely automated these assays such that the technical labor is no longer a disadvantage of the technique. Reagent costs are still substantially higher than conventional techniques, but the ease of performance has substantially reduced technical labor requirements and improved turnaround time.

Any of the methods described can provide clinically useful data. The choice of method depends on a number of factors including total test volume, available personnel, turnaround time requirements, and instrumentation available. Numerous reports now document that the newer EIAs are much more sensitive than electrophoresis or other activity-based assays. Consequently they are able to detect acute MI sooner than other methods. Regardless of the method employed, it is universally accepted that interpretation of isoenzymes cannot be done at a single point in time and is reliable only when serial specimens are viewed simultaneously. Specimens should be collected on admission and then every 8 hours for 24 to 48 hours. A typical rise/fall curve as shown in Fig 1 is diagnostic of acute MI.

There is increasing evidence, however, that with the newer immunoassays sampling intervals should be shortened such that

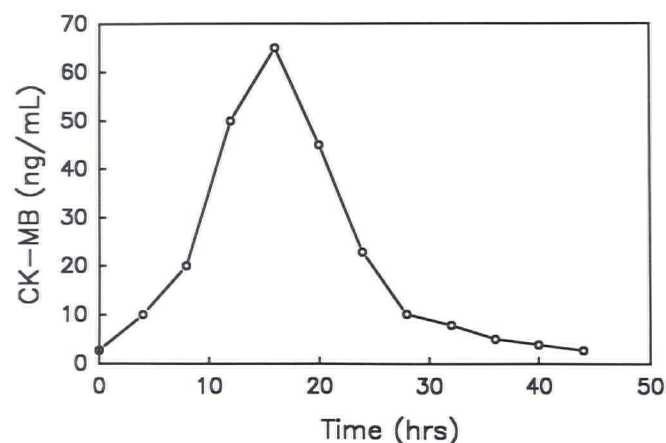


Fig 1—CK-MB concentration in myocardial infarction.

Table 2
Early Detection Capability of Immunoassays*

	Patients Meeting Laboratory Criteria After Just 2 Assays	Patients Meeting Laboratory Criteria Only After 3 Assays
Non-acute MI	96%	4%
Acute MI	100%	0%
Differences Between Assays		
	1 & 2	2 & 3
Non-acute MI	1.1	0.7
Acute MI	52	18

*From Elwart DM, Shimkovitz KL. Minimum number of CK-MB assays necessary to confirm or rule out a diagnosis of acute myocardial infarction. Poster presentation, Michigan Society of Medical Technology, Annual Meeting, May 2, 1991. Used with permission. MI = myocardial infarction.

two or three samples would be drawn in the first 8 hours following the event. Two recent studies have suggested that acute MI can be diagnosed within the first 8 hours using the newer EIA techniques (11,12). As shown in Table 2, CK-MB measured by immunoassays can differentiate acute MI patients from non-acute MI patients much earlier than previously thought possible. These data show that 96% of the patients with acute MI were properly diagnosed with only two specimens drawn at 8-hour intervals. Studies by Gibler et al (11) indicate that diagnosis of acute MI can be made in the first 6 hours after symptom onset using 3-hour sampling intervals. Clearly, the use of immunoassays, such as the Stratus assay used at Henry Ford Hospital, which measure CK-MB mass are preferred to previously employed methods.

Lactate dehydrogenase

LD is a tetramer having a molecular weight of 134,000. The subunits can be of two types, M or H, each under separate genetic control. The resulting combinations produce five different isoenzymes: LD-1 (H₄), LD-2 (H₃M), LD-3 (H₂M₂), LD-4 (HM₃), and LD-5 (M₄). The isoenzymes of LD are often referred to in terms of their electrophoretic mobility. The fastest (toward the anode) migrating enzyme is LD-1 and the slowest is LD-5 when separated in an alkaline medium. A sixth LD isoenzyme, LD-X, has been described. It is composed of four X subunits and is present in postpubertal human testes. Lactate dehydrogenase is widely distributed throughout the body but is found only in the cytoplasm of the cell. It is present in the liver, heart, kidney, striated muscle, red blood cells, and lung tissue. The isoenzymes of LD are somewhat specific in their distribution as shown in Table 3.

Historically, methods for the separation of LD isoenzymes were available much earlier than were procedures for the identification of the CK isoenzymes. Electrophoretic separation and relative quantitation on agarose gels or cellulose acetate membranes is most commonly used. Electrophoretic separation of LD isoenzymes is effective, relatively accurate, and still widely used in many clinical laboratories. Immunological separations have also become increasingly popular. Since there are many

Table 3
Tissue Localization of LD Isoenzymes

Isoenzyme	Tissue
LD-1, LD-2	Heart Kidney Brain Red blood cells
LD-3	Thyroid Adrenal gland Lymph nodes Pancreas Thymus Spleen Leukocytes
LD-4, LD-5	Liver Skeletal muscle

LD = lactate dehydrogenase.

acceptable variations for the electrophoretic separation and quantitation of LD isoenzymes, a somewhat generic procedure is described here. Agarose gels are most popular but cellulose acetate is acceptable. Quantitation is by densitometric scanning with visible or fluorescent detection. Following the separation at pH 8.6 to 8.8, a reaction mixture is layered over the separation medium. The reaction mixture contains L (+) lactate, NAD, and is buffered at pH 9.0 to 9.2. After the reaction has incubated at 37° to 40° C, the plates are dried at 65° C. A fluorescent scanner is used to determine the relative fluorescence following excitation at 340 nm. If a fluorescent scanner is not available, procedures incorporating dye coupled reactions (to NADH) can be used. Fluorescent scanning results in more uniform bands than does visual scanning.

The other widely used approach is based on the precipitation of M-subunit containing LD isoenzymes. This technique allows specific quantitation of the LD-1 or H₄ isoenzyme. It is much simpler than electrophoresis and has a definitive normal range. LD isoenzymes are ordered almost exclusively for the purpose of confirming acute MI. Thus, the fact that other LD fractions are not identified and quantitated is not a serious limitation. The procedure is available from Roche Diagnostics in kit form.

Concern exists over the necessity of evaluating CK and LD isoenzymes simultaneously. Most reports have recommended that both be done. However, recent studies suggest that CK-MB alone is highly sensitive and specific for acute MI (13,14). Asaying LD isoenzymes or LD-1 adds nothing to the diagnostic efficiency of laboratory testing. Laboratory determination of LD isoenzymes may be helpful when specimens for analysis have not been obtained within the first 24 hours following a suspected acute MI.

Testing guidelines

The following testing and interpretation guidelines are relative to cardiac enzyme assays:

If the patient is seen within 24 hours of a suspected event, obtain total CK and CK-MB levels upon admission and then every eight hours for 24 to 48 hours. The admission specimens can be considered baseline levels if obtained within the first 2 hours. If

several positives are observed on successive specimens, the protocol can be discontinued.

If the patient is not seen within the first 24 hours, both CK-MB and LD-1 (or isoenzymes) should be ordered. In this case LD-1 > 85 or LD-1/LD-2 > 1.0 is consistent with the diagnosis of acute MI even in the presence of negative CK-MB.

In cases involving skeletal muscle trauma or crush injuries, CK-MB may be positive but usually will not exceed 3% of the total CK. When using immunoassays which measure mass units, a CK-MB/total CK ratio can be used. At our institution a ratio ≤ 3 is indicative of skeletal muscle injury. The LD-1/LD-2 ratio will be < 1.0 and LD-1 will remain normal, i.e., < 85 IU/L or < 25% of total LD.

In all cases, cardiac enzymes cannot be interpreted on the basis of a single specimen collection. A single positive or negative value is not indicative of the presence or absence of acute MI. A confirmatory diagnosis can only be made using at least two but preferably three specimens serially drawn at 8-hour intervals.

Hemolysis of any source can elevate LD-1. CK-MB will not be affected unless hemolysis is severe and then only if activity assays are used.

Standard testing guidelines such as those described should be followed to insure appropriate management of patients suspected of MI. Table 4 summarizes evaluation criteria of laboratory data for the diagnosis of acute MI. CK-MB mass assays are also reported to be useful in evaluating thrombolytic therapy (15). A rapid rise in CK-MB following the initiation of thrombolytic therapy indicates successful reperfusion. This rapid wash-out of CK-MB is illustrated in Fig 2.

Conclusion

Currently CK-MB is the best marker routinely available for the diagnosis of MI. The new EIAs which measure mass are more sensitive than previously available methods. They are capable of detecting acute MI sooner and have proven to be valuable in the assessment of thrombolytic therapy. Thus, the new EIAs are recommended as the method of choice for CK-MB measurement but with significantly reduced time intervals between analyses.

Of the LD isoenzymes, only LD-1 is of value in acute MI. Specific measurement of LD-1 by immunoprecipitation is the

Table 4
Laboratory Criteria for Diagnosis of Acute MI

To Confirm Acute MI:

- At least one of three consecutive CK-MB assays > 5 ng/mL and CK-MB index > 3, or
- ≥ 50% increase in successive determinations, or
- Rise/fall curve with a peak at 12-20 hours.

To Rule Out Acute MI:

- At least three consecutive negative CK-MB determinations over the first 24-hour period following onset of symptoms, or
- LD-1 < 85 IU/L or < 35% of total LD 24-72 hours after onset of symptoms.

MI = myocardial infarction.

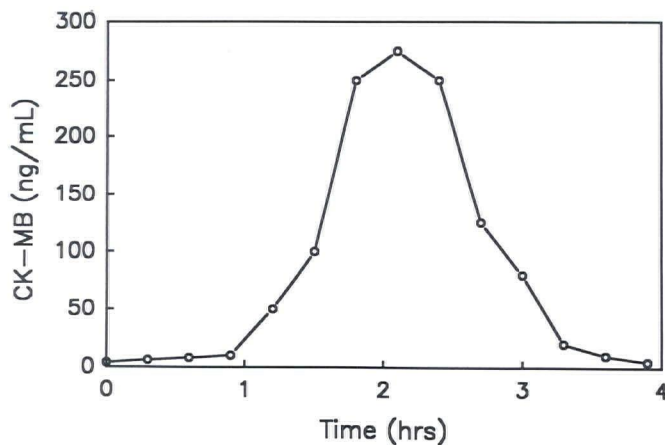


Fig 2—CK-MB following successful reperfusion.

preferred method. LD-1 should only be measured in late admissions.

CK-MM and CK-MB isoforms are reported to have clinical utility in the diagnosis of acute MI, infarct sizing, and assessment of reperfusion. Currently only electrophoretic methods can determine all of the various isoforms, limiting the timely availability of these analyses. Furthermore, the sensitivity of the isoforms has not been demonstrated to be better than EIAs for total CK-MB.

Troponin and myosin light chains are biochemical markers which show promise in the diagnosis and evaluation of acute MI. Troponin appears to be the long sought cardiac-specific assay. Myosin light chains may be of value in assessing infarct size. Commercialization of these methods will allow widespread evaluation of the clinical usefulness of these analyses.

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