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Creatine Kinase MB Isoforms in Patients with Skeletal Muscle Injury: Ramifications for Early Detection of Acute Myocardial Infarction

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We measured total creatine kinase (CK), CK-MB isoenzyme, and the MB isoforms in 202 serum and plasma samples from nine groups of patients and normal individuals: 39 with acute myocardial infarction (MI), divided according to time between the onset of chest pain and blood collection (1–6 h, 7–12 h, and 13–48 h); 26 with chest pain for whom an MI was ruled out, sampled at admission; 17 undergoing bypass surgery or cardiac catheterization, sampled within 6 h after either procedure; 17 with acute skeletal muscle injury, sampled within 8 h after injury; 30 marathon runners immediately after a race; 17 runners and other athletes >12 h after training or a race; 12 with cerebral injury or seizures, sampled at admission; 8 with closed head injury, sampled at admission; and 38 normal subjects. CK-MB (relative index) and MB isoforms (MB_2/MB_1) were respectively increased in 15% and 75% of MI patients 1–6 h after onset, 94% and 94% after 7–12 h, and 88% and 8% after 12 h, and in 87% and 82% of cardiac surgery patients. MB isoforms were increased in most patients with acute skeletal muscle trauma and in subjects examined after exercise, but were within normal limits in patients for whom MI was ruled out, patients with cerebral trauma, and normal individuals. The relative index of MB/total CK was normal in essentially all individuals in the last groups, including those with acute skeletal muscle trauma. We concluded that the CK-MB isoform ratio is increased in both acute skeletal muscle injury and MI. The isoform ratio is most useful for distinguishing recent from old (>12 h) injury.

Additional Keyphrases: *thrombolytic therapy · exercise, effects of · stroke · closed head injury · scleroderma · isoenzymes*

A biochemical marker for early diagnosis of acute myocardial infarction (MI)[5] would be important for patients being considered for thrombolytic therapy because such therapy is best administered within the first 6 h of chest pain (*1, 2*). Several enzymes and proteins have been examined as possible markers for early biochemical diagnosis. Assay of the isoforms of the MM isoenzyme of creatine kinase (CK) has a sensitivity for MI of 58% when blood samples are collected 3–6 h after the onset of chest pain (*3*); assay of myosin light chains has a sensitivity for MI of 65% at 4 h after onset (*4*); and assay of myoglobin has a sensitivity of 72% on samples collected at (average) 3.4 h after pain onset (5). Although studies of myosin light chains I and II were promising initially, subsequent studies showed that within the first day after MI, the increases in these chains parallel those of CK-MB (*6*). Increased concentrations of myoglobin and MM isoforms are seen in acute skeletal muscle injury and may be useful for the exclusion of acute MI (*7*).

Troponin has also been studied as a marker for diagnosis of acute MI (*8*). Assays of myocardial troponin T and troponin I are highly specific for myocardial damage, there being no cross-reactivity with skeletal muscle troponin isotypes (*9, 10*). The value of troponin as an early marker of MI is largely unknown. In one study in which blood samples were collected from 23 MI patients in the emergency room, assay of troponin T had a clinical sensitivity of 57% as opposed to 35% for CK (*9*); however, the time from onset of chest pain to blood collection was not recorded.

Isoforms of CK-MB may provide the best single marker of acute MI because they are released within the 6-h diagnostic window after chest pain and because MB is more specific to the myocardium than is myoglobin (*11*), total CK, or CK-MM isoforms (*3*). The serum of normal individuals contains a mixture of tissue MB (MB_2) and converted serum MB (MB_1) isoforms (*12*). Methods for MB isoform analysis include high-voltage electrophoresis on agarose gel (*13*) and immunoextraction assay with a CK-MB_2-specific antibody (*14*). Using high-voltage electrophoresis, Puleo et al. found that the clinical sensitivity for acute MI was 12.5% at 0–2 h, 59% at 2–4 h, and 92% at 4–6 h after MI onset (*12*). In subsequent studies, these investigators suggested that in non-MI patients, MB isoforms in blood samples collected within 6 h of symptom onset could be used to determine which patients could be transferred from the emergency room to non-coronary-care units (*15*). These studies were conducted on a selected population of patients admitted to the emergency room and coronary-care unit with chest pain. However, the activity of total CK-MB in the blood increases in patients with skeletal muscle trauma or disease and after cardiac surgery.

We examined blood samples from various groups of non-MI patients for total CK, MB, and MB isoforms and compared the results with those for MI patients. Our goal was to determine whether other clinical conditions

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[5] Nonstandard abbreviations: CK, creatine kinase (ATP:creatine *N*-phosphotransferase, EC 2.7.3.2); LD, lactate dehydrogenase (EC 1.1.1.27); and MI, myocardial infarction.

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can produce false-positive results when CK-MB isoforms are used as an indicator for acute MI. We were particularly interested in characterizing the relation between skeletal muscle CK-MB isoforms and the different trauma and disease conditions to determine whether the measurements could be used to produce diagnostic information. Individuals who train and compete in marathon races were studied as a model for skeletal muscle injury, since the onset of damage would be known precisely.

Materials and Methods

Subjects and Specimens

We followed a protocol approved by the University of Texas Health Science Center Committee for the Protection of Human Subjects (HSC-MS-83-001). We obtained plasma from 120 hospitalized patients admitted to the coronary care, surgical intensive care, or neuro-intensive care units. The patients included 24 males (ages 40–99 years) and 15 females (41–83 years) with MI; 19 males (15–86 years) and 7 females (34–75 years) with a diagnosis in which MI that had been ruled out; 9 males (39–69 years) and 8 females (53–76 years) undergoing cardiac surgery (bypass or acute cardiac catheterization); 11 males and 6 females with acute skeletal muscle trauma; 4 males and 8 females with either stroke or seizures; 5 males and 3 females with closed head injury; and 1 female with acute scleroderma.

Diagnoses were made by attending physicians who had no knowledge of CK-MB isoform results. For MI, diagnosis was based on clinical history, electrocardiographic recordings, and changes in serum enzyme activities [total CK, CK-MB, lactate dehydrogenase (LD), and the LD-1 isoenzyme]. Some MI patients were treated with intravenous thrombolytic therapy during their initial hospital course, although none received such therapy before the initial blood collection in the emergency room. Cardiac catheterization was performed on 26 of 39 MI patients within 5 days after MI; acute catheterization was performed on only 7 of these patients within the first 24 h after onset.

We were unable to determine whether any of the trauma patients suffered concomitant cardiac injury; however, in most cases, myocardial involvement was unlikely.

We also obtained plasma from 30 runners (both sexes) within 1 min after completion of a marathon race, from 13 runners at least 12 h after the end of a training session, from 2 runners at 24 h after a race, from 2 members of an Olympic crew team at 24 h after the end of a training session (consisting of hard running), and from 38 normal individuals with no unusual history of extensive exercise.

Blood was collected by venipuncture into red- and purple-top Vacutainer Tubes (Becton Dickinson, Rutherford, NJ), the former containing no preservatives and the latter containing EDTA, 3.8 mmol/L. Samples were centrifuged and the serum was stored at 2–8 °C until analysis. Plasma samples were analyzed for CK-MB isoforms; serum was analyzed for total CK and CK-MB. The integrity of CK-MB isoforms is maintained when the serum carboxypeptidase N activity is inhibited by chelation with divalent cations (*16*). Serum samples were not always available for each plasma sample collected at a given time.

Assays

Total CK activity was assayed with the Hitachi 717 random-access analyzer (Boehringer Mannheim Corp., Indianapolis, IN), with CK-NAC reagents (Boehringer Mannheim) at 37 °C. A reference range of 12–191 U/L was used for both sexes. The mass concentration of CK-MB was measured by using the Stratus II (Baxter Scientific, Miami, FL). We used a reference range of 0–9 μg/L and a relative index, [CK-MB, μg/L/(total CK, U/L)] × 100, of <2.5 (*17*). The cutoff values were established by comparing MB results from patients with confirmed MI with those from non-MI patients with chest pain. Normal healthy subjects were not used in the establishment of the cutoff for MI. CK-MB isoforms were measured by a high-voltage electrophoresis system (Rep; Helena Laboratories, Beaumont, TX) (*12*). We used the ratio 1.4 for MB_2/MB_1 as a cutoff value for the diagnosis of MI. Although other investigators using the Rep system have reported using a cutoff value of 1.5 (*14*), we used a slightly lower value to optimize clinical sensitivity. CK-MB concentrations <1.0 μg/L were undetectable on the Rep system and were considered normal.

Results

CK-MB Isoforms in Normal Individuals

Total CK activity of 38 normal individuals showed a mean of 130 U/L (SD 30) with a range of 9 to 393. CK-MB concentrations in 23 of these individuals had a mean of 2.0 μg/L (SD 1.1) with a range of <0.4 to 3.2. The CK-MB isoform ratio in 18 of 38 normal individuals had a mean of 1.00 with a range of 0.33–1.33. In the other 20 plasma samples, the CK-MB isoform concentrations were below the detection limit for the assay.

CK-MB Isoforms in Cardiac Diseases and Injury

Figure 1 shows the results of total CK-MB and MB isoforms for MI patients with blood samples collected 1–6, 7–12, and 13–48 h after the reported onset of chest pain. Total CK was increased in 55% (11 of 20), 88% (15 of 17), and 96% (24 of 25) of the samples, respectively. For CK-MB, the clinical sensitivity for these groups was 15% (3/20), 94% (16/17), and 88% (22/25), respectively. For the MB isoform ratios, the corresponding sensitivities were 75% (15/20), 94% (15/16), and 8% (2/25), respectively; furthermore, the sensitivity was 67% (8/12) in the 1–3 h interval, and 87% (7/8) in the 3.5–6 h interval. In contrast, the relative CK-MB index was only 16.6% (2/12) and 12.5% (1/8) positive in these time groups, respectively.

CK-MB isoenzyme and isoform results in patients with coronary artery diseases and in patients for whom an MI was subsequently ruled out are also tabulated in Figure 1. The clinical specificity is nearly equivalent at

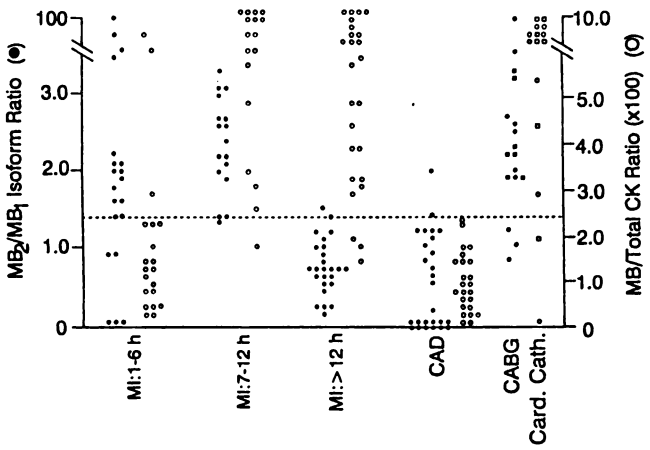

Fig. 1. Distribution of relative index for CK-MB and MB isoform ratios
Results are for patients with acute MI, divided according to time between the onset of chest pain and blood collection; patients after coronary artery bypass surgery (CABG, circles) and cardiac catheterization (squares); and patients with coronary artery disease (CAD), for whom an MI was ruled out

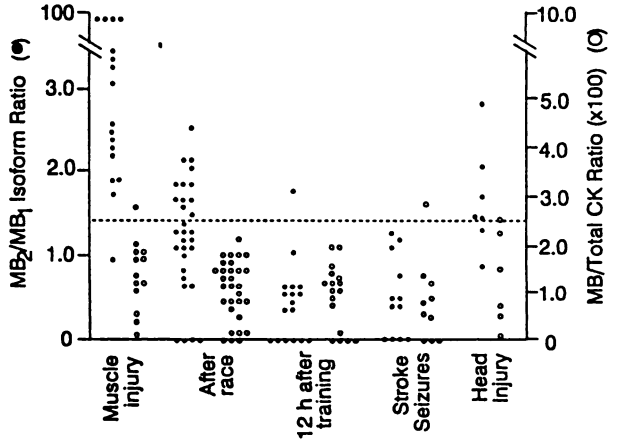

Fig. 2. Distribution of relative index for CK-MB and MB isoform ratios
Results for patients with acute skeletal muscle injury, trained athletes immediately after a marathon race, athletes >12 h after intense training or a race, patients with cerebral stroke or seizures, and patients with closed head injury

100% (24/24) for the MB isoenzyme and 92% (24/26) for MB isoforms.

Results for blood samples collected from patients within a few hours of either coronary artery bypass surgery or cardiac catheterization showed that, immediately after either procedure, CK-MB isoenzymes and isoforms were abnormal [87% (13/15) and 82% (14/17), respectively] (Figure 1).

CK-MB Isoforms in Skeletal Muscle Injury

Although all patients with acute skeletal muscle injury had absolute increases in total MB (results not shown), only 1 of 12 patients (8%) had an abnormal relative index (Figure 2). In contrast, the MB isoform ratio was abnormal in all but 1 of 17 patients (94%) with acute skeletal muscle injury.

To characterize further the release of MB after skeletal muscle injury, we obtained blood from men and women after each had run a marathon race. The blood was obtained between 2 and 3.5 h after the start of the race. As shown in Figure 2, 40% (12/30) of the runners exhibited abnormal MB isoform ratios. For blood samples collected 20 h after a race and samples collected >12 h after a training session, MB isoform ratios were normal. We also examined one patient with scleroderma. This individual, who was seen because of a recurrence of acute symptoms, had a normal total CK (142 U/L) and high values for MB (relative index = 38.2) and MB isoform ratio (2.30).

CK-MB Isoforms after Seizure, Stroke, or Head Injury

Although total CK was increased in 67% (4 of 6) of stroke patients and 67% (4 of 6) of patients with seizures, MB isoforms were increased in none (Figure 2). For total CK-MB, only one patient had an increased relative index: the absolute MB concentration, however, was 2.2 μg/L, which is below our 9 μg/L cutoff limit.

We also examined serum samples from patients with traumatic closed head injury (Figure 2). Total CK was increased in 5 of 6 of these patients, but only 1 of 6 had increased CK-MB. Increased concentrations of CK-MB isoforms were found in 5 of 7 patients.

Discussion

This study was conducted to verify the utility of measuring MB isoforms for early detection of acute MI and to demonstrate that acute release of the MB_2 isoform is not unique to instances involving damage to the myocardium. Our data show the superiority of the use of MB isoforms over the use of total CK and CK-MB for diagnosis of MI between 1 and 6 h, particularly between 3.5 and 6 h. The results are consistent with previous reports (*14*). This study also shows that some acute myocardial injury accompanies cardiac surgery, as reflected in the release of MB isoenzymes (*18*) and MB isoform. Cardiac surgery produces some myocardial injury, whether or not MI has occurred. Although perioperative MI is difficult to diagnose by means of cardiac enzyme testing, recent studies based on mass measurements and a high cutoff value of 75 μg/L have shown that CK-MB may be a useful marker (*19*). Our review of the medical records did not indicate postoperative MI in any of the patients. We did not determine whether MB isoforms can be used as an aid in the diagnosis of perioperative MI or whether they indicate postoperative injury. However, others have shown that measurements of MM isoforms are not effective indicators of these conditions (*20*).

The data from patients with cerebral injury are consistent with the observation that the brain contains little or no CK-MB (*21*). Some of the increased activity of total CK may be caused by the release of CK-BB (*22*) and mitochondrial CK (*23*). Because cerebral injury alone should not increase MB concentrations and because many of these patients also had concomitant skeletal muscle injury, the abnormal CK-MB isoform concentrations probably originated from skeletal muscle turnover. It is possible, however, that in some of these patients, cardiac injury was the result of trauma. The diagnosis of such injury is difficult with serum markers alone. Nevertheless, the acute release of MB

isoforms in these patients does not occur to the extent that measured values would exceed the cutoff of the relative MB index, and thus differentiation can be made by use of both indicators.

Total CK-MB is not useful for early diagnosis of MI because there is a residual amount of MB present in serum as a result of normal skeletal muscle turnover. To be effective in the first few hours after acute MI, the amount of MB released from the heart must significantly exceed the amount already present. CK-MB isoforms (MB_2) in serum can indicate MI at an early stage because their serum concentrations are normally low. The myocardium contains only the MB_2 isoform, and any increase of this isoform is the result of recent necrosis (*24*). Because restoration of the normal ratio of MB_2 to MB_1 requires several hours, our data on MI patients show that the utility of isoform measurements is in the differentiation between release before vs after 12 h.

In contrast, the CK-MB isoform ratio cannot be used to differentiate between damage to the skeletal muscle and damage to the myocardium. We examined MB isoforms in patients with traumatic injury because skeletal muscles contain small amounts of MB (*25*). Our data show that patients and individuals with acute muscle injury can have abnormal concentrations of MB_2 similar to those observed in acute MI. Marathon runners also had increased amounts of tissue isoforms immediately after a race. For these patients with high total CK, measurement of the relative CK-MB index by a sensitive immunoassay is most useful for detecting skeletal muscle injury because the relative amount of MB is less than that seen in MI (*26, 27*). However, in patients with concomitant skeletal muscle and myocardial injury, the diagnosis of MI is difficult with any of the CK markers, and test results should be interpreted with caution (*28*).

The kinetics of enzyme release in marathon runners and MI patients is similar: acute skeletal muscle injury results in the release of the tissue isoform of MB beginning ~3 h after the onset of injury. If the injury is not ongoing, conversion to CK-MB_1 (serum form) is largely complete after 12 h. None of the runners, either in training or after a marathon race, showed a relative index for total MB that was above normal limits measured with a specific immunoassay. These results differ from those of earlier electrophoresis studies, which showed that the relative amount of CK-MB was increased in the serum of highly trained marathon runners (*29*).

We did not measure CK-MB isoforms in patients with chronic skeletal muscle diseases such as the muscular dystrophies. On the basis of our present data, we would expect the MB isoform ratio to be normal in these patients unless they were experiencing an acute exacerbation of the illness. However, previous data showed a decrease in the tissue MM isoform ratio of polymyositis patients whose clinical course was deteriorating (*30*).

In Figure 3 we summarize the data presented herein.

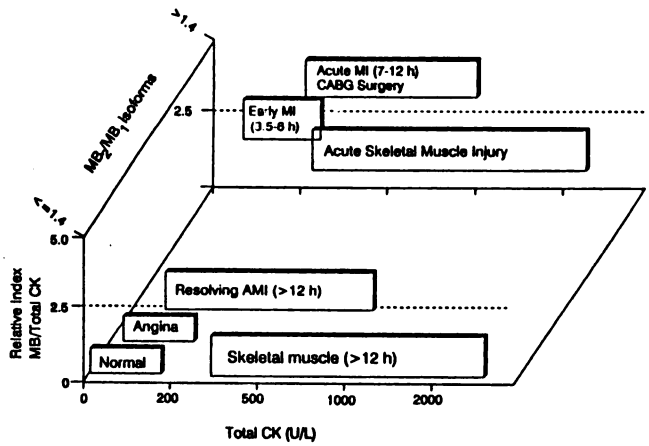

Fig. 3. Summary of expected serum results for total CK (*x*-axis), CK-MB relative index (*y*-axis), and CK-MB isoforms (*z*-axis) in normal individuals and patients with various diseases

Healthy individuals had low total CKs and normal MB indices and isoform ratios. Non-MI patients had slightly higher values for these markers, but results were below the cutoff values. Blood collected from MI patients between 1 and 6 h (and particularly between 3.5 and 6 h) after onset was normal for CK and CK-MB and abnormal for the MB isoform ratios. Blood collected after 12 h was abnormal for total CK and MB, but normal for MB isoforms. Patients with acute skeletal muscle injury had increased total CK, absolute CK-MB, and MB isoforms, but had a normal relative CK-MB index. Patients with skeletal muscle injury of >12 h duration had abnormal total CK and CK-MB with a normal relative MB index and isoform ratio.

References

1. Schwarz F, Schuler G, Katus H, et al. Intracoronary thrombolysis in acute myocardial infarction: duration of ischemia as a major determinant of late results after recanalization. Am J Cardiol 1982;50:933–7.
2. TIMI Study Group. The thrombolysis in myocardial infarction (TIMI) trial. N Engl J Med 1985;312:923–6.
3. Wu AHB, Gornet TG, Wu VH, et al. Early diagnosis of acute myocardial infarction by rapid analysis of creatine kinase isoenzyme-3 (CK-MM) sub-types. Clin Chem 1987;33:359–62.
4. Jackowski G, Symmes JC, Wu TW, Mickle DAG. Rapid early diagnosis of myocardial infarction and unstable angina utilizing a unique myosin light chain assay. Circulation 1989;30:854.
5. Schulz A, Larsen CE, Kristensen SD, et al. Serum myoglobin measured by latex agglutination: rapid test for exclusion of acute myocardial infarction. Am Heart J 1986;112:609–10.
6. Wang J, Shi Q, Wu TW, et al. The quantitation of human ventricular myosin light chain 1 in serum after myocardial necrosis and infarction. Clin Chim Acta 1989;181:325–36.
7. Konnigs CH, Funke Kupper AJ, Verheugt FWA. Comparison of two latex agglutination test kits for serum myoglobin in the exclusion of acute myocardial infarction. Ann Clin Biochem 1989; 26:254–8.
8. Gerhardt W, Katus H, Ravkilde J, et al. S-troponin T in suspected ischemic myocardial injury compared with mass and catalytic concentrations of S-creatine kinase isoenzyme MB. Clin Chem 1991;37:1405–11.
9. Mair J, Artner-Dworzak E, Lechleitner P, et al. Cardiac troponin T in diagnosis of acute myocardial infarction. Clin Chem 1991;37:845–52.
10. Bodor G, Porter S, Ladenson JH. Human cardiac troponin I measurement in suspected myocardial infarction with a double

monoclonal antibody sandwich ELISA [Abstract]. Clin Chem 1990;36:1103.
11. Gibler WB, Gibler CD, Weinshenker E, et al. Myoglobin as an early indicator of acute myocardial infarction. Ann Emerg Med 1987;16:851–6.
12. Puleo PR, Guadagno PA, Roberts R, Perryman MB. Sensitive, rapid assay of subforms of creatine kinase MB in plasma. Clin Chem 1989;35:1452–55.
13. Harker CC, Wu AHB. Early diagnosis of acute myocardial infarction (MI) upon initial hospital admission using CK-MB_2 isoform analysis [Abstract]. Clin Chem 1990;36:1128.
14. Puleo PR, Guadagno PA, Roberts R, et al. Early diagnosis of acute myocardial infarction based on assay for subforms of creatine kinase-MB. Circulation 1990;82:759–64.
15. Puleo PR, Wathen C, Roberts R, Perryman MB. Triage of patients with chest pain in the emergency room guided by MB creatine kinase subform assay [Abstract]. J Am Coll Cardiol 1992;19:303A.
16. Chapelle JP, Bertrand A, Heusghem C. The protection of creatine kinase MM sub-bands by EDTA during storage. Clin Chim Acta 1981;115:255–62.
17. Wu AHB, Gornet TG, Harker CC, Chen HL. Role of rapid immunoassays for urgent ("stat") determination of creatine kinase MB. Clin Chem 1989;35:1752–6.
18. Graeber GM. Creatine kinase (CK): its use in the evaluation of perioperative myocardial infarction. Surg Clin North Am 1985;65: 539–51.
19. Gulbis B, Unger P, Lenaers A, et al. Mass concentration of creatine kinase MB isoenzyme and lactate dehydrogenase isoenzyme 1 in diagnosis of perioperative myocardial infarction after coronary bypass surgery. Clin Chem 1990;36:1784–8.
20. Keshgegian AA, Bhagat PK, Lease JA, Balocchi J. Creatine kinase MM isoforms in serum after cardiac surgery. Clin Chem 1988;34:1067–70.
21. Neumier D. Tissue specific distribution. In: Lang H, ed. Creatine kinase isoenzymes. Pathophysiology and clinical applications. Berlin: Springer Verlag, 1981:85–109.
22. Schwartz JG, Bazan C, Gage CL, et al. Serum creatine kinase isoenzyme BB is a poor index to the size of various brain lesions. Clin Chem 1989;35:651–4.
23. Liu N, Wu AHB, Friedman DL, Hamburg RJ, Perryman MB. Detection of serum mitochondrial creatine kinase in cerebrovascular accident patients [Abstract]. Clin Chem 1992;38:984.
24. Panteghini M, Cuccia C, Malchiodi A, et al. Isoforms of creatine kinase MM and MB in acute myocardial infarction: a clinical evaluation. Clin Chim Acta 1986;155:1–10.
25. El Allaf M, Chapelle J-P, El Allaf D, et al. Differentiating muscle damage from myocardial injury by means of the serum creatine kinase (CK) isoenzyme MB mass measurement/total CK activity ratio. Clin Chem 1986;32:291–5.
26. Thompson WG, Mahr RG, Yohannan WS, Pincus MR. Use of creatine kinase MB isoenzyme for diagnosing myocardial infarction when total creatine kinase activity is high. Clin Chem 1988;34:2208–10.
27. Koch TR, Mehta UJ, Nipper HC. Clinical and analytical evaluation of kits for measurement of creatine kinase MB. Clin Chem 1986;32:186–91.
28. Bruns DE. Diagnosis of acute myocardial infarction when skeletal muscle damage is present: a caveat regarding use of creatine kinase isoenzymes [letter]. Clin Chem 1989;35:705.
29. Siegel AJ, Silverman LM, Holman L. Elevated creatine kinase MB isoenzyme levels in marathon runners. JAMA 1981;246:2049–51.
30. Annesley TM, Strongwater SL, Schnitzer TJ. MM subisoenzymes of creatine kinase as an index of disease activity in polymyositis. Clin Chem 1985;31:402–6.