Unusual Findings Related to Atypical Creatine Kinases in Two Hospitalized Patients

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We describe two cases, hospitalized patients, in whom the activity of creatine kinase (EC 2.7.3.2) isoenzyme-MB was above normal. Both are particularly noteworthy in that creatine kinase-BB and a macro creatine kinase form thought to be type II of mitochondrial origin were also present. The macro creatine kinase component in both cases co-migrated electrophoretically with creatine kinase-MM but was easily identified after the latter was removed by precipitation with M-subunit-specific antibodies. In the first case, the patient had a readily diagnosable acute myocardial infarction while under observation in the cardiac intensive care unit: electrocardiographic changes and the rapid increase and decrease in total creatine kinase were as would be expected. In marked contrast, in the second case, we saw no abrupt changes in either of these characteristics. The latter patient’s primary disease was a rectal carcinoma with massive metastases to the liver; however, the presence of abnormally high creatine kinase-MB activity raised the question of possible myocardial infarction.

An increase in the MB isoenzyme of creatine kinase (CK-MB) in serum is now generally accepted as one of the best markers for a myocardial injury (1). However, depending upon the method used, the concurrent presence of the CK-BB isoenzyme and other atypical forms will tend to diminish the clinical specificity for acute myocardial infarction. The presence of CK-BB in heart muscle (2-4), in the plasma of patients in acute coronary care units (5), and after open heart surgery (5, 6) has been reported (see ref. 7 for a more complete review). There has also been an increasing number of reports on patients with atypical creatine kinases, either macro CK type I, which is usually IgG-linked CK-BB (3, 9), or macro CK type II, which is similar in many of its properties to mitochondrial CK (10-14). We report here two cases in which activities of total CK and CK-MB were increased to values seen in acute myocardial infarction, but in which the interpretation of laboratory results was not straightforward, owing to the concomitant presence of two other forms, CK-BB and macro CK type II.

Materials and Methods

Serum samples were analyzed for total CK by a procedure recommended by the International Federation of Clinical Chemistry (15); the B-subunit estimates of CK-MB by immunoinhibition (“Cardiozyme Plus”; Harleco Division, E. Merck, Gibbstown, NY 08027) (16); the analytically more specific CK-MB measurement by immunoprecipitation with use of “Isomune CK” antibodies 1 and 2 (Roche Diagnostics, Nutley, NY 07110); and electrophoresis in the “Paragon System” (Beckman Instruments, Brea, CA 90621) before and after immunoprecipitation (17). Aspartate and alanine aminotransferases (ASAT and ALAT, respectively), alkaline phosphatase, and lactate dehydrogenase were measured by methods developed for a National Bureau of Standards cooperative enzyme study group (18).

Case I

This 85-year-old white woman who had a history of hypertension and irregular heart beat entered the emergency room in acute respiratory distress. Her admitting diagnoses were pulmonary edema, pneumococcal pneumonia, congestive heart failure, and atherosclerotic heart disease. A mass was noted in the right lower quadrant, but there were no signs or symptoms of metastatic bone or liver disease. Two days after admission to the cardiac intensive care unit for observation, progressive enzyme changes (Figure 1) and electrocardiographic changes, both consistent with an acute inferior and anterolateral myocardial infarction, were observed. Her cardiac and respiratory problems responded to conservative medical therapy and she was discharged to a nursing home after three weeks of hospitalization.

During this period of hospitalization, the pattern of an acute, rapid increase and subsequent decrease in total CK activity was paralleled by a similar rise and fall of CK-MB activity as determined by: (a) B-subunit estimate by immunoinhibition, and (b) electrophoretic analysis. However, the proportion of B-subunit (34%) in the initial immunoinhibition test values was abnormally high for an infarction and suggested the presence of either CK-BB or an atypical CK, or both. Subsequent electrophoresis at different intervals (Figure 2) confirmed the presence of CK-MB, CK-BB, and an atypical CK that was co-migrating with CK-MM but that

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Fig. 1. Time course of serum CK and LDH activities in Case I

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was detectable only after CK-MM had been removed by immunoprecipitation. This atypical CK fraction was not visible in the electrophoretogram of a specimen drawn 50 h after admission but was clearly evident at 60 h and increased to its maximum by 110 h. CK-BB was not visible at 50 h after admission but was clearly present at 60 h, and was almost invisible by 86 h and thereafter.

Four of the specimens analyzed by the immunoinhibition technique were also subjected to immunoprecipitation. CK-MB determined by the latter technique rapidly increased to 27 U/L at 62 h after admission and accounted for 10% of the total CK. The difference in the results by the two techniques (Figure 1) appears to be largely accounted for by free CK-BB in the 60- to 62-h period (Figure 2) and by atypical CK migrating with CK-MM in the later specimens (at 86 and 110 h).

LDH activity was sharply increased 60 h after admission, lagging slightly behind the increase in CK. It peaked at fourfold the upper normal reference limit at 86 h, and then returned slowly toward the pre-60-h value. ASAT activity (not shown) increased to approximately fivefold the upper normal reference limit 74 h after admission but by the time of discharge had returned to the value at the time of admission. ALAT and alkaline phosphatase activities (not shown) remained within reference limits throughout the hospital stay.

Six months later, this patient was readmitted for recurrent pulmonary edema and congestive heart failure. There were no signs, symptoms, electrocardiographic changes, or enzyme activity changes suggestive of an acute myocardial infarction. The diagnostic evaluation revealed abnormal results of liver-function tests, results by abdominal CT scan and ultrasonography consistent with massive hepatic metastases, and palpable cystic pelvic masses consistent with ovarian neoplasms. Results of mammography and a barium enema were negative. The final laboratory tests from this admission showed a slightly increased value for total CK (138 U/L), and CK-MB by immunoinhibition as 291 U/L (209% of the total CK). CK-MB by immunoprecipitation was undetectable. Electrophoresis was not performed; therefore, the identity of the B-subunit activity is uncertain. Because the patient refused further studies, a tissue diagnosis was not obtained. She died three months later at home; an autopsy was not performed.

Case II

This patient, a 54-year-old white man with a long history of cigarette smoking and alcohol abuse, was admitted for evaluation of jaundice and abdominal pain. Physical examination revealed massive liver enlargement, and a large rectal tumor mass. Laboratory tests documented marked hyperbilirubinemia, anemia, and the above-normal enzyme values shown in Table 1. One examiner suggested a diagnosis of alcoholic hepatitis, but a senior gastroenterology consultant stated unequivocally that the overall picture, including results of the liver-function tests, was most consistent with metastatic liver disease. The high CK-MB value caused a house officer to suggest the possibility of an acute myocardial infarction, but at no time during this admission were there any symptoms or electrocardiographic evidence of this. Furthermore, the time course of both the total CK and CK-MB failed to show the sharp peak of activity characteristic of infarction. The patient's condition, which precluded biopsy, deteriorated steadily and he died on the eleventh day after admission. Autopsy confirmed the presence of rectal carcinoma with massive metastases to the liver. There was minimal coronary atherosclerosis and, although an old focal site of myocardial fibrosis was seen, there was no gross evidence of a recent myocardial infarction.

As shown in Table 1, the CK-MB activity evaluated by immunoinhibition exceeded total CK activity in most measurements. The presence of CK-BB and (or) atypical forms was signaled by the very high calculated values for CK-MB by immunoinhibition and by the much lower results by immunoprecipitation. Electrophoresis of several of these specimens revealed a pattern very similar to what was seen at 60 and 62 h in Case I (Figure 2), with distinct bands of CK activity in positions corresponding to the MM, MB, and BB isoenzymes. Much of the large band migrating to the CK-MM position remained after immunoprecipitation. This band was not adenyate kinase (EC 2.7.4.3), because it was no longer present when an identical prepared strip was treated with CK reagent from which substrate had been deleted. However, mitochondrial CK, isolated from fresh heart tissue by the method of Wevers et al. (13), migrated to the same position; therefore, we assumed that this band was atypical CK of the macro CK type II from mitochondria.

<table>
<thead>
<tr>
<th>Day after admission</th>
<th>Enzyme activity, U/L</th>
<th>CK-MB*</th>
<th>Immunoinhibition</th>
<th>Immunoprecipitation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Alk. phos.</td>
<td>ASAT</td>
<td>ALAT</td>
<td>LDH</td>
</tr>
<tr>
<td>1</td>
<td>1500</td>
<td>185</td>
<td>50</td>
<td>934</td>
</tr>
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<td>3</td>
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<td>11</td>
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<td>154</td>
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<td>1434</td>
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</table>

*% of CK<sub>total</sub> is indicated in parentheses. The immunoinhibition results have been corrected for residual adenyate kinase activity, which never exceeded 11 U/L.
The prominent CK-MB band present after electrophoresis of untreated serum disappeared after immunoprecipitation of the specimen (17), but the CK activity in the CK-BB position was unaffected. Other enzyme activities (Table 1) also exceeded their normal upper reference limits throughout most of this patient's hospital stay, and there were no dynamic increases and decreases in any of these enzyme activities to suggest an acute myocardial infarction. Significantly, activities of other enzymes of hepatic origin were grossly increased: alkaline phosphatase (EC 3.1.3.1) and ASAT exceeded their upper reference limits by 15- and 8.5-fold, respectively.

Discussion

In each of these patients the increased total CK and CK-MB so often associated with acute myocardial infarction was seen; also increased, however, were CK-BB and an atypical macro CK type II, which are not associated with this disorder. Although the values for total CK were similar at 60 and 62 h in Case I to those seen in Case II on day I, other clinical and laboratory findings differed markedly and pointed to completely separate pathological processes.

In Case I, the patient, while under observation in the cardiac intensive care unit, had a "silent" infarction, documented by progressive changes in the electrocardiogram and a dynamic pattern of enzyme changes. Both total CK and the CK-MB activity increased and returned predictably to a steady state in a pattern that was completely compatible with an acute myocardial infarction. Furthermore, ASAT and LDH test results were consistent with an infarction. In Case II, no such changes in total CK or in CK-MB were noted; in fact, there were no clinical or laboratory indicators of an infarction other than the increased CK-MB. The normally high but slightly declining values for alkaline phosphatase and ASAT, as well as the hyperbilirubinemia, persisted to the end.

Although it is very unlikely that the Case II patient had an infarction, we nevertheless think that the portion of CK determined as MB after immunoprecipitation analyses was the authentic MB form. Not only did a band occur in the correct position during electrophoresis, but also the band was no longer visible on electrophoresis after precipitation with antibody specific for the M-subunit. Thus the CK-MB demonstrated in these two cases is clearly different from the "false" CK-MB reported by others (19–26). The source of this CK-MB is not obvious to us, but a similar appearance of CK-MB has been reported with various different tumors (1, 27–30), in patients with no evidence of an acute myocardial infarction. Hybridization between the CK-MM normally present and CK-BB from tumor is certainly one possibility, and such hybridizations have been reported in vitro (31, 32) and in vivo (7).

In Case I, serial electrophoresis of samples before and after the acute myocardial infarction (Figure 2) affords a qualitative picture of the CK activity represented by the discrepancy between immunoinhibition and immunoprecipitation results (Figure 1). This activity appears to be divided mainly between CK-BB and macro CK type II. Appearance of CK-BB immediately after an infarction (60 and 62 h) and its replacement by increasing amounts of macro CK type II in the 86- to 134-h period are events presumably associated with heart damage. However, because both forms are also characteristic markers of tumor (7, 8, 19, 26, 29, 33), the source of CK-BB and macro CK type II in Case I is in doubt. At least one other report (34) describes a patient with a malignant tumor who, during hospitalization, had an acute myocardial infarction and in whom laboratory studies showed high CK-MB and CK-BB activities at the time of the infarct and a rapid decrease of these two isoenzyme activities in the next three days. Likewise, macro CK type II reportedly is present in sera of patients with cardiac disorders (10) and other diseases (25, 35), as well as in the sera of cancer patients (24, 25, 36, 37).

In our laboratory, the incidence of macro CK forms or of free CK-BB, relative to analyses of all CK isoenzymes, is about 3%. However, these infrequent occurrences can cause considerable confusion, particularly when they involve patients who have had or are suspected of having had an acute myocardial infarction. To screen our CK-MB results more carefully, we currently rerun by the more specific immunoprecipitation assay all samples in which the percent CK-MB (by immunoinhibition) exceeds 14% of total CK. Previously, this re-analysis was performed only when the CK-MB percentage exceeded 20% (17). Only rarely must we include electrophoresis to resolve a more complex isoenzyme picture, as in the two cases described here. In such cases, the presence of macro CK type II co-migrating with CK-MM can be easily unmasked by including an immunoprecipitation step before electrophoresis.

The two cases in this report demonstrate how the proper clinical as well as laboratory interpretation of CK-MB results involves the integration of all historical, physical, and laboratory information. The explanation for an increase in CK-MB should be consistent with the clinical diagnosis and not simply equated to myocardial necrosis.

References

15. EPE/IFCC methods for the measurement of the catalytic concentration of enzymes, Part 7; IFCC method for creatine kinase (ATP:creatine phosphotransferase, EC 2.7.3.2). To be published.