Clinical Evaluation of Immunoinhibition Determination of Creatine Kinase B Subunits in Coronary Care

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One hundred patients with chest pain of cardiac origin were evaluated on the basis of clinical findings, electrocardiograph results, and total creatine kinase (CK) and creatine kinase B-subunit (CK-B) activity (as determined by immunoinhibition with the Boehringer CK-MB kit) in serum. All patients diagnosed as having had an acute myocardial infarction had increased values for both CK-B and total CK. In no case was normal total CK activity associated with an increased CK-B, nor was normal CK-B associated with an increased total CK. During collection of data for reference ranges, we found 10 patients who had no evidence of cardiac disease but had various other diseases, who exhibited high values for CK-B in serum; four of these had normal values for total CK. We conclude that estimations of CK-B in serum by this method added no more diagnostic information than did data on total CK in the evaluation of chest pain.

Additional Keyphrases: "kit" methods · reference interval · myocardial infarction

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Traditionally, enzymic evaluation of acute myocardial infarction (AMI) is based on the activities in serum of total creatine kinase (CK; EC 2.7.3.2), aspartate aminotransferase (EC 2.6.1.1), and lactate dehydrogenase (EC 1.1.1.27). These assays are sensitive tests for the diagnosis of AMI but unfortunately are not specific, because each of the three enzymes may be increased in serum in various other conditions. The "heart" isoenzyme of creatine kinase, CK-MB, is highly specific for myocardial damage and is an excellent test for the assessment of AMI (J.2). However, the traditional laboratory methods for estimation of this isoenzyme (electrophoresis, chromatography) are technically difficult and time consuming. Recently, determination of the CK B-subunit (CK-B) in serum by immunoinhibition has become available, and many reports of its value in the diagnosis of AMI have appeared in the literature (3–5). The concentration of CK-B in serum is estimated by a simple immunological technique and, when chest pain is present, any increase in CK-B is considered to be due to an increase in CK-MB and thus indicates myocardial damage. The laboratory estimation of this subunit is simple, and several commercial

References

kits for doing so have been marketed.

In this study we evaluated the clinical usefulness of determinations of the serum CK-B subunit. We used the Boehringer immunoinhibition kit in the diagnosis of chest pain and compared its diagnostic effectiveness with that of measuring total serum CK.

Materials and Methods

Patients. All patients with chest pain consistent with myocardial ischemia who were admitted to the coronary care unit over a period of three months were included in the study. Their ages varied from 50 to 81 years. Concentrations of total CK and CK-B in the serum were estimated twice daily (0700 and 1900 hours) for three and five days.

Diagnosis. The diagnosis of AMI was made by two cardiologists on the basis of the World Health Organization criteria (6), which require at least two of the following: (a) a history of chest pain consistent with myocardial ischemia; (b) electrocardiographic changes consistent with AMI, i.e., appearance of Q waves or, in the absence of Q waves, S-T segments or T-wave changes associated with typical serum enzyme (total CK) changes; and (c) a value for total CK twice the upper reference limit or an increase to a value of at least threefold the initial value. By these criteria, 29 patients were classified as AMI and 71 as non-AMI.

Enzyme assay. We measured the CK-B subunit with the Immunoinhibition Boehringer Kit (CK-MB NAC-act; Boehringer Mannheim GmbH Diagnostica, Mannheim, F.R.G.). We also measured total CK with a kit method (CPK activated; Boehringer Mannheim Diagnostica).

Reference intervals. For the purpose of this study we derived reference intervals for total CK and CK-B from 130 healthy preoperative patients, ages 50–80 years. All patients with renal insufficiency, malignancy, or a history of myocardial or skeletal muscle disease were excluded. The upper reference values (x + 2 SD) for total CK and CK-B were 150 and 17 U/L, respectively.

Results and Discussion

Figure 1 shows plot of peak values for total CK vs the peak values obtained for CK-B for the two groups of patients. Table 1 lists the diseases and serum enzyme values for 10 patients discovered to have increased CK-B values during the course of gathering information for the enzyme reference intervals. None of these 10 patients had any clinical evidence of myocardial or skeletal muscle disease.

As Figure 1 shows, the CK-B values clearly differentiated these clinically preselected AMI patients from non-AMI patients (AMI ≥20 U/L, non-AMI ≤20 U/L). However, it is also clear that the CK-B values offer no great advantage over total CK values. Because CK-B is more specific for cardiac muscle necrosis, perhaps values exceeding the upper limit of the reference interval (17 U/L) indicate substantial myocardial necrosis. If this were the case, five patients diagnosed here as non-AMI would have their diagnosis changed. However, all of these five cases had normal or equivocal electrocardiographic patterns, and all but one (whose total CK was 155 U/L) had values for total CK that were within the reference interval. These factors suggest that muscle necrosis in these five patients, if any, was minimal. Marmor et al. (2) suggest that ischemic myocardial cells that have not progressed to necrosis may leak enzymes, thereby somewhat increasing their concentrations in serum; these five cases may fall into this category.

A major problem of relying on CK-B values for the diagnosis of AMI is that an increase of the enzyme subunit in serum is not specific for myocardial damage. Both increased CK-MB (cardiac isoenzyme) and increased CK-BB (brain isoenzyme) will have the same effect. Moreover, increased concentrations of CK-B in serum have also been described in patients with head injury (7), prostatic carcinoma (8), dermatomyositis (9), or breast cancer (10), and patients on hemodialysis (11). During our investigation we found increased CK-B in several patients who had no clinical evidence of cardiac disease (Table 1).

Table 1. Total CK and CK-B Values in 10 Preoperative Patients Who Had No Clinical Evidence of Cardiac or Skeletal Muscle Disease

<table>
<thead>
<tr>
<th>Disorder</th>
<th>CK, U/L</th>
<th>CK-B subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic carcinoma</td>
<td>106</td>
<td>93</td>
</tr>
<tr>
<td>Bronchogenic carcinoma</td>
<td>110</td>
<td>40</td>
</tr>
<tr>
<td>Colonic carcinoma</td>
<td>683</td>
<td>305</td>
</tr>
<tr>
<td>Colonic carcinoma</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td>Disseminated carcinoma</td>
<td>111</td>
<td>32</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>681</td>
<td>358</td>
</tr>
<tr>
<td>Myeloma, renal failure</td>
<td>533</td>
<td>49</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1466</td>
<td>22</td>
</tr>
<tr>
<td>Sepsis</td>
<td>814</td>
<td>71</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1250</td>
<td>22</td>
</tr>
</tbody>
</table>

We conclude that serum CK-B values, determined here with the Boehringer immunoinhibition kit, have no advantage over serum total CK values in the evaluation of chest pain of cardiac origin. They may be useful in evaluating those patients with chest pain who already have high total CK concentrations, if other causes of increased CK-B can be excluded.

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References

Enzyme Immunoassay for Urinary Albumin

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We describe an enzyme-linked immunosorbent assay for urinary albumin, performed on microtiter plates with use of commercially available antisera and peroxidase conjugate. The assay range is 3–1000 µg/L, the sensitivity 625 pg. The method is suitable for measurement of albumin excretion in either normal or pathological urine. For 20 normal children, the range of urinary albumin excretion was 1.7–22.9 mg/24 h.

Additional Keyphrases: proteins • pediatric chemistry • enzyme-linked immunosorbent assay

Following the advent of more sensitive techniques for the assay of urinary albumin, there has been increased clinical interest in differentiating subtle abnormalities in urinary albumin excretion. For example, exercise-induced albuminuria has been measured to define early glomerular changes in diabetic subjects (1, 2), and patterns of albumin and low-molecular-mass protein clearance after renal transplantation have been studied (3, 4).

Most current methods for sensitive albumin measurement are radioimmunoassay (5–7) involving 125I-labeled albumin. Disadvantages of using this reagent include its short shelf life, its associated health hazards, and the expense of equipment used to measure gamma-emitting isotopes. Radical immunodiffusion (8) and electroimmunoassay (9) have been used but are rather cumbersome and slow. An immunoturbidimetric assay has recently been reported (10), but this would not be sufficiently sensitive to measure the lowest concentrations of urinary albumin in healthy children as reported in this paper.

In response to the need for a simple assay sensitive enough to distinguish normal concentrations of urinary albumin from slightly increased concentrations, in a range sufficiently large to accommodate excretion rates in pathological states, we have developed an enzyme-linked immunoassortent assay (ELISA) for the measurement of urinary albumin. In common with other ELISA techniques (11, 12), this procedure overcomes many of the disadvantages of radioimmunoassay. It is sufficiently sensitive to measure accurately albumin at low concentrations, is easily set up in a laboratory, and is very simple to carry out.

Materials and Methods

Reagents. Human transferrin and immunoglobulin G (IgG), o-phenylenediamine, Tween 20, and bovine serum albumin were obtained from Sigma Chemical Co., Ltd., London, U.K. The following antisera and peroxidase conjugate were obtained from Miles Laboratories Ltd., Slough, U.K.: rabbit anti-human albumin (lyophilized), cat. no. 65-051; goat anti-human albumin (liquid), cat. no. 61-015; and horseradish peroxidase (EC 1.11.1.7) conjugated to anti-goat IgG (heavy + light chain), cat. no. 61-201. Inactivated rabbit serum was obtained from the Wellcome Foundation Ltd., London, U.K.

Buffers. A solution of phosphate-buffered saline and Tween 20 (PBS-Tween) was prepared by dissolving 7.88 g of Na2HPO4 • 2H2O, 2.04 g of NaH2PO4 • 2H2O, 9.00 g of NaCl, and 0.5 mL of Tween 20 in 1 L of distilled water.

Diluent was prepared by dissolving 2.5 g of bovine serum albumin per liter of PBS-Tween. At this concentration, the bovine serum albumin did not cross react with the antisera in the assay system but was sufficient to inhibit nonspecific binding.

The coating buffer (0.5 mol/L carbonate/bicarbonate buffer, pH 9.6) was made by dissolving 1.59 g of Na2CO3, 2.93 g of NaHCO3, and 0.20 g of NaN3 in 1 L of distilled water.

The substrate solution was prepared just before use: to 25 mg of o-phenylenediamine in 100 mL of 0.2 mol/L phosphate solution, pH 6.0 (28.6 g of Na2HPO4 in 1 L of distilled water), 160 mL of 300 g/L hydrogen peroxide solution was added. The substrate solution was prepared in and dispensed from a brown bottle.

Standards. Human albumin, dissolved in the diluent solution to give a stock solution of 200 mg/L, was kept at −20 °C. From this, standards were made up in diluent at concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and

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