Creatine Kinase: Aspartate Aminotransferase Activity Ratio as an Indicator of the Source of an Increased Creatine Kinase Activity

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Although measurements of creatine kinase isoenzyme 2 (CK-MB) are often used to diagnose acute myocardial infarction, their sensitivity and specificity are <100%. Because skeletal muscle contains more CK and less aspartate aminotransferase (AST) than cardiac muscle, the CK/AST ratio might provide a useful adjunct in evaluating the source of a supranormal value for CK. I established the following decision levels in a retrospective study of 342 patients: ratios <14 (if total CK was 300–1200 U/L), <20 (CK 1201–2000 U/L), or <25 (CK >2000 U/L) suggested myocardial infarction, with a sensitivity of 95% and a specificity of 65%. In a validation study with 277 additional patients, liver disease and alcohol abuse caused erroneous results, leading to exclusion of 22% of these patients. In the remaining cases, sensitivity was 94%, specificity 90%. The CK/AST ratios changed little with time, suggesting that a single value would be adequate for evaluating patients with increased CK.

Additional Keyphrases: heart disease • myocardial infarction • cutoff values • alcohol abuse • liver disease • causes of increased CK

The laboratory tests most utilized for the diagnosis of acute myocardial infarction are creatine kinase (CK, EC 2.7.3.2) and its MB isoenzyme (CK-MB, CK-2) (1, 2). Although measurement of CK-MB has been suggested to be the "gold standard" for the diagnosis of myocardial infarction (3), various other, non-cardiac disorders may also cause increases of CK-MB (4–7). An increase in CK-MB is commonly considered to have a sensitivity of 99–100% in patients with myocardial infarction (2, 3); however, a recent study demonstrated that normal myocardium may be devoid of CK-MB (8). Thus, in some instances, it may be necessary to utilize other laboratory tests to establish the correct diagnosis.

Measurement of aspartate aminotransferase (AST, EC 2.7.1.1) was the first laboratory procedure used to diagnose myocardial infarction. In recent years, its determination for this purpose has largely been abandoned (2), although one study suggests that AST is the single best discriminating test for evaluating patients with acute chest pain (9). Myocardium contains considerably less CK, but more AST, than skeletal muscle (10). Given these observations, Garcia-Webb et al. (11) postulated that a high ratio of CK/AST would be expected with skeletal muscle damage, whereas a low ratio would indicate myocardial infarction. In comparing patients with postoperative increases of CK with patients with myocardial infarction, they found that use of a CK/AST ratio of 11.0 would distinguish the two groups. In a

1 Nonstandard abbreviations: CK, creatine kinase; CK-MB, creatine kinase isoenzyme 2; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK/AST, ratio of CK activity to AST activity.
limited prospective evaluation of patients admitted to a coronary-care unit, they calculated that the ratio had a sensitivity of 94% and a specificity of 96%, but found the ratio unreliable at extremely high values for total CK activity.

Unfortunately, their study did not address several issues regarding the usefulness of the CK/AST ratio. The cause of skeletal muscle damage, the effect of co-existing liver disease, the nature and magnitude of time-dependent changes in the ratio, and the prospective validation of the ratio in unselected patients are issues that require study before the CK/AST ratio can be considered for routine use. In an attempt to answer these questions, I derived decision levels (cutoff values) for the CK/AST ratio from a retrospective study of 342 patients with increased CK, and studied the factors affecting the ratio in an additional 277 consecutive patients with increased CK.

**Materials and Methods**

**Patients**

To establish decision levels for the CK/AST ratio, I identified all patients who had increased CK and for whom simultaneous AST measurements were available for the interval from March 1, 1983, to December 31, 1986. The cause of the increased CK was determined by review of hospital records. Of the total of 342 patients who met the criteria, 130 had acute myocardial infarction and 212 had skeletal muscle damage.

To evaluate the usefulness of the ratios, I then reviewed all results of CK determinations made between January 1987 and April 1988 and identified 277 patients with increased CK: 79 had acute myocardial infarction and 198 had skeletal muscle damage. The distribution of the causes of the skeletal-muscle damage was similar to that seen in the first part of the study (Table 1).

**Methods**

Total CK was measured at 37 °C in a centrifugal analyzer (Centrifichem 600; Baker Instruments, Pleasantville, NY 10570; or Monarch; Instrumentation Laboratory, Lexington, MA 02173) by a coupled enzymatic reaction with N-acetylcysteine activation (CK-NAC reagent; Roche Diagnostic Systems, Nutley, NJ 07110). AST and alanine aminotransferase (ALT, EC 2.7.1.2) were measured at 37 °C in a SMA-2 continuous-flow analyzer, by coupled enzymatic reactions, with reagents supplied by the manufacturer (Technicon Instruments Corp., Tarrytown, NY 10591). Reference intervals for CK were established by using initial specimens from patients who were admitted to the intensive-care unit with stable angina or chest pain of non-cardiac origin. The upper reference limit was 300 U/L for black men and 230 U/L for white men. In the prospective study, AST and ALT activities were measured in all non-hemolyzed specimens from patients with increased CK for whom enough leftover serum was available. In patients with suspected myocardial infarction, specimens were routinely obtained on admission and 8 and 16 h later (specimens 1, 2, and 3, respectively). In patients with skeletal-muscle disease, specimen 1 was the specimen obtained at the time of admission; specimens 2 and 3 were obtained 8 to 24 h later. The ratio CK/AST was determined by dividing the activity of CK by that of AST.

**Results**

On the basis of receiver-operating characteristic curves (Figure 1), I derived the following decision levels for the CK/AST ratio from the retrospective analysis of results from 342 patients: ratios >14 (if total CK was <1200 U/L), >20 (if total CK was between 1200 and 2000 U/L), and >25 (if total CK exceeded 2000 U/L) suggested skeletal-muscle damage. These ratios collectively had a sensitivity of 98% for acute myocardial infarction and a specificity of 65% in patients with skeletal muscle origin for CK. In this retrospective study, results for ALT were not routinely available for most of the patients, so further subclassification of the data was not attempted.

When I used these decision levels in a validation study involving results from 277 additional patients with increased CK, the sensitivity for the group as a whole was 95% and the specificity was 62%, similar to the performance of the ratio in the retrospective study. I next analyzed the data for cause of CK increase in patients with skeletal-muscle disease. As Figure 2 shows, patients with alcohol abuse had low CK/AST ratios, indistinguishable from those in patients with myocardial infarction. Because hepatocellular necrosis causes increases in both AST and ALT activities in serum, co-existing liver disease might be expected to cause falsely low CK/AST ratios in patients with skeletal-muscle disorders. In patients with liver disease, as recognized by an increased ALT, the CK/AST ratios were similar to those seen in patients with myocardial infarction (Figure 2).

I then re-analyzed the data for CK/AST ratio, excluding the 22% of patients with documented increased ALT or

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**Table 1. Noncardiac Causes of Increased CK**

<table>
<thead>
<tr>
<th>% of total noncardiac causes</th>
<th>Retrospective (n = 212)</th>
<th>Prospective (n = 198)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myopathy</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Alcoholic</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Seizures</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Surgery</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Trauma*</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>Unknown</td>
<td>27</td>
<td>28</td>
</tr>
</tbody>
</table>

*Includes cardiopulmonary resuscitation.

b Includes data from two unpublished studies on increased CK in pulmonary disease and pancreatitis.

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**Fig. 1. Receiver–operating characteristic curve derived from values obtained for 342 patients with increased total CK, used to select optimal decision levels for the CK/AST ratio**

A, the cutoff value of 11 suggested by Garcia-Webb et al. (17); B, a single cutoff value of 14. Points C and D represent use of multiple cutoff points: at point C, two different cutoff values were used (14 for total CK <1200 U/L, >20 for total CK >1200 U/L); at point D, including a third cutoff value of 25 for total CK >2000 U/L improves sensitivity with minimal change in specificity. E, a single cutoff value of 25 for all activities of CK, shows a marked decrease in specificity.
documented alcohol abuse. In the remaining 78% of the patients, the sensitivity of the ratio for myocardial infarction was 94%, the specificity in patients without myocardial infarction was 90%, and the diagnostic efficiency of the ratio was 92%.

For 83 patients, multiple determinations of the CK/AST ratio were available; the changes in this ratio with time are illustrated in Figure 3. In patients with myocardial infarction, the CK/AST ratio was stable or decreased over the first 8–16 h of hospitalization in 36 of 40 patients; even when still increasing, it changed from diagnostic to non-diagnostic in only one patient. In patients with skeletal-muscle disease, the ratio increased in 20 of 43 patients, decreasing from a high ratio to a ratio suggesting myocardial infarction in only five patients.

**Discussion**

The diagnosis of myocardial infarction often depends greatly on laboratory data, because many patients lack a typical history or evidence of electrocardiographic changes. Measurements of CK-MB have thus been advocated as the "gold standard" for diagnosis of myocardial infarction. The specificity of CK-MB for myocardial damage is not absolute, however, because skeletal muscle also contains CK-MB (12). In chronic myopathic disorders, regenerating skeletal-muscle cells often revert to production of the fetal isoenzyme, CK-MB (13), with a resulting higher myofibrillar content of CK-MB (14). Even using a ratio of CK-MB to total CK activity of >5% as a diagnostic criterion yields a specificity for myocardial infarction of only 95–97% (2). The sensitivity of CK-MB for myocardial infarction is also <100%, probably because of intra-individual and regional variations of myocardial CK-MB content (15) and the possibility (raised in an unconfirmed report) that CK-MB may not be present in normal myocardium (6). In unpublished data from this institution, only 91% of patients with acute myocardial infarction who presented within 24 h of the onset of symptoms had CK-MB of >5% of total CK activity. Thus, additional tests may be necessary for evaluating patients with suspected myocardial infarction.

Skeletal muscle contains approximately five times as much CK per gram of tissue as does cardiac muscle, but only about half as much AST (10). To exploit these differences, Garcia-Webb et al. (11) proposed the use of the ratio of CK to AST as a possible diagnostic test in patients with an increased CK. In 62 patients with myocardial infarction and 25 postoperative patients, a ratio <11 occurred only in the myocardial infarction patients, whereas a ratio >11 typified the postoperative patients (11). They then tested the ratio in 99 patients admitted to the coronary-care unit, and found that the sensitivity was 96% and the specificity 94%. They also found this single ratio to be unreliable in patients whose values for CK were either very high or within the normal reference interval. Although they did not evaluate further, they cautioned that the ratio would probably not be useful in patients with liver disease, and questioned its usefulness in patients with causes of CK increases other than surgery or myocardial infarction.

In the current study, I have extended their observations and validated the usefulness of the CK/AST ratio. However,
I found that the optimum decision level increased as the total CK increased. The likely explanation for this increase is the "background" created by the basal AST value. If, for example, the true ratio of CK to AST in a given skeletal muscle is 40:1, then damage to muscle causing an increase in serum CK activity of 400 U/L would be expected to increase serum ALT activity by 10 U/L. If the basal CK is 180 U/L and basal AST 20 U/L (median values of the reference ranges), then the CK/AST ratio would be 900/40, or 24.

As predicted by Garcia-Webb et al. (11), the CK/AST ratio was of little use in patients with co-existing liver disease, as defined by a history of alcohol abuse or increased serum ALT. In these patients, AST is derived from both muscle and liver, and the resulting CK/AST ratios are similar to those seen in patients with myocardial infarction. In our hospital, where the frequency of liver disease and alcohol abuse is high, 22% of all samples thus were excluded from further analysis. Interestingly, however, only 8% of patients with myocardial infarction were excluded from analysis because of high ALT values or history of alcohol abuse. Obviously, this is a major limitation to the use of the CK/AST ratio, and appears to be a problem regardless of the cause for liver damage. It adversely affects test specificity, but does not increase false-negative results; thus, a ratio above the decision levels could still be used to exclude myocardial infarction even in the presence of liver disease. In other settings, where the frequency of liver disease is not as high as in this institution, the frequency of patient exclusion may not be as great.

Except for the patients with liver disease, the CK/AST ratio was useful for patients with trauma, seizures, myopathies, postoperative patients, and in persons with unexplained increases of CK. In addition, through the use of multiple decision points, the ratio was equally effective at all values of CK increases above the reference interval. In fact, its usefulness increased with increasing total CK, with most of the false-positive results occurring in patients with total CK between one and two times the upper reference limit (probably reflecting the background effect noted above).

An unanswered question from the previous study is how the duration of the CK increase affects the CK/AST ratio. If it were to change significantly with time, its usefulness could be limited. In the present study the ratio was stable or decreasing during the first 24 h of hospitalization in patients with myocardial infarction, and stable or increasing in patients with skeletal-muscle damage. In only 7% of these patients would the interpretation of the CK/AST ratio have changed with time. Thus, the result for a single specimen appears to accurately reflect the type of muscle damaged. The reasons for a decrease in CK/AST ratio with time in patients with myocardial infarction is not clear, because the reported clearance rates for CK and AST after myocardial infarction are similar (10). Perhaps the presence of mitochondrial AST after myocardial infarction is involved. Mitochondrial AST represents ~20% of total AST released after a myocardial infarction (16). This isoenzyme has a significantly longer half-life than cytoplasmic AST, and remains increased in the serum for at least six days after an infarction (17). Because the half-life of mitochondrial AST is longer than that of CK-MB, the CK/AST ratio may prove useful in patients presenting more than 24 h after onset of chest pain. This idea will require validation by further study, which is currently underway. In this series only a few patients with myocardial infarction were studied more than 24 h after the onset of chest pain; in all of them, the CK/AST ratio was in the diagnostic range for myocardial infarction. These data suggest that the ratio is useful within the first 24–36 h after onset of symptoms in patients with myocardial infarction, but are insufficient to support its use beyond this time frame.

The actual decision levels for CK/AST are likely to vary between laboratories, because different methods for measurement of enzyme activity give different results. Garcia-Webb et al. (11) reported a decision level of CK/AST of 11, but their upper reference limit for CK of 180 U/L is considerably lower than ours. It will be necessary for other investigators to establish appropriate decision levels derived from the CK and AST measurements performed in their laboratories.

On the basis of these data, I conclude that the CK/AST ratio is useful for evaluating patients with increased CK. In this laboratory, the CK/AST ratio has an efficiency equal to CK-MB measurements in determining the source of an increase in CK in patients without co-existing liver disease. It is not helpful in most patients with alcohol abuse or liver disease. Because CK and AST measurements are readily available in most hospital laboratories, this ratio may prove to be a valuable adjunct in the rapid evaluation of patients with chest pain, and may supplement measurements of CK-MB in patients with an equivocal diagnosis.

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Fully Automated Liquid-Chromatographic Determination of Amino Acids
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This inexpensive method for fully automated amino acid analysis combines the advantages of automated precolumn derivatization with o-phthalaldehyde and favorable analytical conditions to separate and quantify 30 amino acids found in normal plasma. The system can run unattended for almost four days, during which the data are processed automatically by a personal computer and a maximum of 76 samples and 19 standards can be processed (cycle time per analysis: 55 min). Only 1 μL of deproteinized plasma is required per analysis. Coefficients of variation for retention times and areas measured for all relevant amino acids are less than 1% and 3%, respectively. The system described is well suited for quick, sensitive operation in daily practice.

Metabolic or clinical studies requiring multiple analyses of amino acids are generally hindered by the fact that amino acid determinations are relatively expensive and time consuming. Many methods for determining amino acids in biological samples involve precolumn derivatization with o-phthalaldehyde (OPA), followed by high-performance liquid chromatography (1-11). None, however, satisfactorily separates as many as 30 amino acids, including all the prominent amino acids in plasma, and has fully automated precolumn derivatization and data analysis. We describe a system that does have these features and can run unattended for 87 h. During this period, 76 samples and 19 standards are processed.

Materials and Methods

Equipment. Our HPLC system consists of two pumps (both Model 2152), a Model 2152 controller, a Model 2156 solvent conditioner, and a Model 2155 column oven, all from LKB, Woerden, The Netherlands. The separation column is a Bischoff Spherosorb ODS II column (3-μm particles, 25 cm × 4.6 mm (i.d.), equipped with a 10 mm × 4.6 mm (i.d.) guard column filled with the same packing material (Salm & Kipp, Breukelen, The Netherlands). For automated precolumn derivatization we use a WISP 7125B sample processor (Millipore/Waters, Etten-Leur, The Netherlands), equipped with a cooled sample-storage compartment. To monitor fluorescence we use a fluorescence detector equipped with a xenon lamp and a 12-μL flow cell. Measurements are made at an excitation wavelength of 330 nm and an emission wavelength of 445 nm.

Data are collected on-line by a Model 763 SB 192 K interface (Nelson Analytical, CLI, Schijndel, The Netherlands) and processed by an Olivetti M24SP personal computer (Woltink, Kerkrade, The Netherlands) utilizing Nelson Analytical software.

Reagents and solvents. We use "ultra-pure" water, processed with a Milli-Q system (Millipore/Waters). All chemicals used are of analytical grade (primarily from Pierce Chemical Co., Oud Beijerland, The Netherlands). Solvents are of chromatographic grade (mostly from Merck, Amsterdam, The Netherlands). The derivatization reagent is prepared by dissolving 12.5 mg of OPA in 0.25 mL of methanol, then adding 2.25 mL of potassium borate buffer (1.0 mol/L, pH 10.4) and 10 μL of 3-mercaptopropionic acid. Solvent A is a 12.5 mmol/L phosphate buffer, pH 7.0, containing 9 mL of tetrahydrofuran per liter. Solvent B consists of the phosphate buffer, acetonitrile, and tetrahydrofuran (53/40/7 by vol). Amino acid standards are prepared by dissolving pure amino acids in water to give a final concentration for each of 250 μmol/L. These are then calibrated against (a) acidic and neutral amino acid standards and (b) basic amino acid standards by using a Model 4400 amino acid analyzer (LKB) with lithium buffers as specified in the LKB operator's manual (12). These calibrations require a 2.5-h run.

Sample preparation. Heparinized blood samples are immediately centrifuged at 1500 × g in a Sorvall GLC-2 centrifuge for 10 min at 4 °C. Plasma is deproteinized with sulfosalicylic acid, 4 mg per 100 μL of plasma, and centrifuged for 10 min at 10 000 × g in an Eppendorf 5414 centrifuge at 4 °C.

To obtain data on "normal" plasma amino acid concentrations in humans, we sampled blood from 30 healthy human volunteers in the postabsorptive (overnight fast) and fed state (1 h after an unspecified lunch). Samples and standards were stored at −70 °C; before analysis, we diluted them 100-fold.

Chromatographic conditions. During the first 4.6 min of the run, the sample processor executes the automated precolumn derivatization. First, 5 μL of the OPA reagent is injected into its sample loop, where it remains because the flow rate is still zero. One minute later, the sampler injects 5 μL of a sample into the loop, at which point the flow rate is slowly increased to 1.2 mL/min, thus allowing sample and reagent to mix and react for 2 min before the gradient elution starts.

The gradient is shown in Table 1.

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