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Plasma Creatine Kinase/Aspartate Aminotransferase Ratio in the Diagnosis of Acute Myocardial Infarction

To the Editor:

During the last 25 years, much has been written on the usefulness of measuring plasma enzyme activity in the diagnosis of myocardial infarction. A recent editorial in this journal (1) concluded that the presence of a "flip" in the ratio of lactate dehydrogenase (EC 1.1.1.27) isoenzymes 1 and 2 together with demonstration of the MB isoenzyme of creatine kinase (CK, EC 2.7.3.2) remained the most sensitive and specific indicators of acute myocardial damage (1). There seems no reason to disagree with this view.

However, facilities for isoenzyme measurement may not be present in smaller laboratories, particularly those in countries other than the United States. Such laboratories would benefit from a more simple way to differentiate between skeletal and heart muscle as the source of increased CK activity in plasma.

Skeletal muscle contains more CK and less aspartate aminotransferase (AST, EC 2.6.6.1.1) per gram than does heart muscle. It therefore follows that the ratio of CK to AST for skeletal muscle exceeds that ratio for heart muscle. With this in mind, we have looked at the usefulness of the plasma CK/AST ratio in the diagnosis of myocardial infarction.

Creatine kinase was measured at 37 °C in a centrifugal analyzer, with creatine phosphate as the substrate and hexokinase (EC 2.7.1.1) and G6PD (EC 1.1.1.49) as indicator enzymes (Sclavo Diagnostics, Siena, Italy; normal reference interval 30–180 U/L (2). AST activity was measured at 37 °C in a SMA II AutoAnalyzer, with ketal-ketoglutarate and aspartate as substrates and malate dehydrogenase (EC 1.1.1.37) as the indicator enzyme (method no. SG4-001U81; Technicon Instruments Corp., Tarrytown, NY; normal reference interval 4–42 U/L). Pyridoxal phosphate was not added. The CK/AST ratio was only tested on those samples in which the CK activity exceeded 200 U/L.

We measured CK and AST activities in plasma samples from 62 patients with proven acute myocardial infarction and from 25 patients who had recently undergone surgery but had not had an acute myocardial infarction. As a separate study, we assessed the sensitivity and specificity of the ratio in a group of 99 patients admitted to a coronary-care ward with suspected myocardial infarction. The diagnosis of acute myocardial infarction was made by the presence of an appropriate clinical history, electrocardiographic changes, and the demonstration of an increase in CK-MB. Samples were collected 12 to 24 h after surgery or admission.

The CK/AST ratio lay between 2 and 11 in the 62 subjects with proven myocardial infarction but was at all times greater than 11 (observed range 11–35) in the 25 post-surgery non-myocardial infarction group (Figure 1). Thus, if the CK activity in plasma was >200 U/L, a ratio of CK to AST of 11 clearly distinguished those patients with myocardial infarction from those with skeletal muscle damage but no infarct.

Of the 99 patients admitted to coronary care, 31 were diagnosed as having had a myocardial infarction. Using the criteria of a plasma CK of >200 U/L plus a CK/AST ratio of 2 to 11, the specificity and sensitivity for the diagnosis of myocardial infarction were 96% and 94%, respectively. The overall efficiency was 95%. There were two patients diagnosed clinically as having a myocardial infarction in whom the CK activity in plasma was <200 U/L (199 and 185 U/L) although the CK/AST ratios were between 2 and 11. These two patients were counted as false negatives.

Obviously, patients with liver disease may have increased plasma AST activities and patients with various types of muscle disease or myopathy may have increased plasma CK activity. The ratio may therefore not be reliable in such clinical settings. In addition, the ratio does not seem to be useful in patients with definite myocardial infarction where the total CK activity is very high (>3000 U/L) as a result of skeletal-muscle damage following cardioversion.

Clearly, one must not rely on the CK/AST ratio alone in making the diagnosis of acute myocardial infarction. Nevertheless, particularly in the small laboratory, we feel that the ratio does have clinical usefulness as a simple test to help in distinguishing patients with myocardial infarction from those with abnormally high CK activity of skeletal muscle origin in their plasma.

References
2. Meiattini F, Giannini G, Tarli P. Ade-

Fig. 1. Relation between creatine kinase and aspartate aminotransferase activity in samples of plasma.

- = patients with myocardial infarction, O = patients post-surgery without myocardial infarction. The solid line indicates a CK/AST ratio of 11:1.
A Fluorescent Biological Compound That Can Cause Error in Enzymic Triglycerides Determination

To the Editor:

Triglycerides in serum can be determined enzymically by continuous-flow analysis after hydrolysis into fatty acids and glycerol by a lipase/esterase combination. The glycerol thus formed is dialyzed and measured by using three enzymic reactions:

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\text{Glycerol} + \text{ATP} + \text{H}_2\text{O} \xrightarrow{\text{glycerokinase}} \text{glycerol-3 phosphate} + \text{ADP}
\]

\[
\text{ADP} + \text{phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + \text{ATP} + \text{H}_2\text{O}
\]

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\text{Pyruvate} + \text{NAD} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}^+
\]

The change in NADH concentration, usually measured spectrophotometrically at 340 nm or by spectrophuorometry (excitation: 340 nm; emission: 460 nm), is proportional to the glycerol concentration. The necessary reagents are commercially available (Triglycerides-Boehringer Mannheim, cat. no. 166448).

We noted spurious results when we used spectrophuorometric detection: very low or zero concentrations of triglycerides in sera from patients with chronic renal failure being treated by hemodialysis. We wondered if these effects could be explained by the presence of fluorescent compounds in the sera. Thus we studied serum from 50 hemodialyzed patients, 25 patients with chronic renal failure treated conservatively, and 50 control patients hospitalized for neurological problems without any apparent renal disease.

The following tests were carried out:

- A search for serum compounds that were fluorescent or that absorbed at 340 nm, with both spectrophuorometric and spectrophotometric detection. We did this by measuring the fluorometric signal given under the same operating conditions as in the assays either when glycerokinase was omitted or reagents were replaced with distilled water.
- Determination of triglyceridemia by the continuous-flow method, with spectrophuorometric or spectrophotometric detection, and by a manual spectrophotometric method (340 nm). In this technique the absorbance of an interferring compound at 340 nm is taken into account by a “blank” serum, glycerokinase being omitted (Triglyceride Test Combination—Boehringer Mannheim, kat. no. 1285012).

With the continuous-flow method four control patients out of 50, 12 chronic renal failure patients out of 25, and 41 hemodialyzed patients out of 50 showed serum fluorescence with spectrophuorometric detection. If we compare the amplitude of the peak with that for the glycerol standards, it is possible to calculate the decrease in apparent triglyceridemia ascribable to these compounds: by from 0.04 to 0.40 mmol/L in control patients, 0.02 to 0.44 mmol/L in chronic renal failure patients, and 0.02 to 2.64 mmol/L with hemodialyzed patients.

With spectrophotometric detection, we saw absorption at 340 nm in only one control patient and those nine hemodialyzed patients who showed the highest fluorescence. The diminution in triglyceridemia was less, corresponding to values ranging from 0.05 to 0.15 mmol/L.

The values for triglycerides obtained with the continuous-flow method are smaller than those obtained with the manual method including a blank. The greatest losses were observed in the case of fluorescent sera measured by the continuous-flow method with fluorometric detection. Table 1 gives the triglyceridemia values obtained by the three techniques for the 10 most fluorescent sera from hemodialyzed patients. Despite two obviously deviating values (patients 1 and 2: zero triglyceridemia), it should be noted that the values considered pathognomonic with the manual method (patients 3, 4, and 6) are considered normal with the spectrophotometric detection method.

The unidentified fluorescence was studied in sera from 12 hemodialyzed patients before and after extrascorporeal dialysis. We noted a diminution of the fluorescence after dialysis; on average it represented 55% of the initial fluorescence while at the same time creatininemia was decreased by 51%.

The fluorescence spectrum shows that excitation is maximum at 322 nm and emission is maximum at 424 nm, characteristics that did not change in the presence of sulfuric acid (50 mmol/L). It is reversibly inhibited by the action of iodine or alkali. The fluorescent compounds are not extractable into chloroform or diethyl ether.

Collectively, these results demonstrate an important source of error in the determination of triglycerides by the continuous-flow spectrophotometric method with some sera, an error that is even greater when the fluorescence of the serum is high.

A comparison of fluorescence spectra of sera and NADH does not lead to the choice of another emission wavelength to make interference negligible.

This potential error is found not only in sera from hemodialyzed and chronic-renal-failure patients, but also in the control patients, although less frequently and to a lesser degree.

We conclude that the spectrophotometric method is not acceptable for use in determining triglycerides in serum. With spectrophotometric detection at 340 nm, interference is much less and is only found in the hemodialyzed patients.

There are several reports of a highly fluorescent compound, probably linked to albumin, in the serum of patients with chronic renal failure and hemodialyzed patients, interfering in the fluorometric assay of isoenzymes of lactate dehydrogenase (1) and creatine kinase (2–7). This compound has not yet been identified, but some of its properties have been described. It is dialyzable and its fluorescence spectrum resembles that of NADH.

The characteristics that we have described resemble those of the fluorescent compound described by Schwertner (6) in serum, urine, dialysis fluids, and hemofilters. According to this author, the fluorescence spectrum shows a maximum excitation at 322 ± 4 nm and a maximum emission at 415...