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Effect of Elevated Concentration of Alkaline Phosphatase on Cardiac Troponin I Assays

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Troponin I is the regulatory subunit of troponin complex associated with the actin thin filament within muscle cells. Cardiac troponin I (cTnI) is a good marker for diagnosis of myocardial damage. Several immunoassays are available for determination of cTnI in serum. The Stratus cTnI fluorometric enzyme immunoassay (Dade International) uses alkaline phosphatase (ALP) substrate. The microparticle enzyme immunoassay (MEIA) for cTnI (Abbott Laboratories) also uses ALP conjugate. On the other hand, the chemiluminescent assay (CLIA) for cTnI (Bayer Diagnostics) does not use ALP. ALP activity may frequently be elevated in serum of patients being evaluated for suspected myocardial infarction. Therefore, we studied the potential interference of ALP in cTnI assays. Serum pools were prepared from patients, and various concentrations of ALP solution were added to different aliquots. The cTnI concentrations were measured by the Stratus, MEIA, and CLIA assays. We observed no interference of ALP in the MEIA and CLIA assay for cTnI. On the other hand, we observed significant positive interference of ALP when cTnI concentrations were measured using the Stratus. J. Clin. Lab. Anal. 15:175–177, 2001.

Key words: cardiac troponin I; alkaline phosphatase; interference

Cardiovascular disease is the major cause of morbidity and mortality in the U.S. Atherosclerosis underlies virtually all cases of myocardial infarction. The precipitating event is often an acute occlusion of a coronary vessel from thrombosis or subintimal hemorrhage into an atherosclerotic plaque. The dying myocardial cells release their content into the cardiac lymphatics and the bloodstream. Therefore, increased concentrations of cardiac enzymes such as creatinine kinase and lactate dehydrogenase can be detected in the peripheral blood after an episode of myocardial infarction and can be used for diagnostic purposes. Creatinine kinase isoenzymes (CK-MB) have been used for diagnosis of myocardial infarction for a long time, but this marker suffers from some serious problems. Individuals with a low total CK may have myocardial infarction (1,2). This is increasingly apparent with recognition of patients with non Q-wave infarction as a subset of patients who were formerly classified within the unstable angina category (3). Concentrations of CK-MB can also be falsely elevated after use of certain drugs like morphine and in alcoholics. Elevated CK-MB concentrations in long-distance marathon runners 1, 24, 48, and 72 hr post run have also been reported. This is due to release of CK-MB from the skeletal muscles of the marathon runner (4).

Troponin I is the regulatory subunit of the troponin complex associated with the actin thin filament within muscle cells. Troponin I in conjunction with troponin C and troponin T plays an important role in the regulation of muscle contraction. Three distinct tissue specific isoforms of troponin I have been identified from skeletal and cardiac muscle. The cardiac troponin I which is 40% dissimilar to the skeletal muscle isoform contains 31 additional amino acids (5,6). Recently, troponin I immunoassays are commercially available. Guest et al. published a report on 209 complex patients to determine the incidence and effect of unrecognized cardiac injury in critically ill patients (7). Guest et al. concluded that troponin I is the best marker because it is found only in heart muscle. Moreover, troponin I is not expressed in response to skeletal muscle injury. Therefore, troponin I concentrations are not falsely elevated in patients with acute or chronic muscle disease, after endurance exercise or renal failure where CK-MB fraction is falsely elevated. Other recent publications also indicate that troponin I concentration is not falsely elevated in hypothyroidism, where CK-MB fraction is elevated (8). Moreover, uremia and skeletal muscle trauma do not lead to

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increase in troponin I concentrations where CK-MB fraction again can be falsely elevated (9–12).

Several immunoassays are commercially available for determination of troponin I concentrations in serum or plasma. The microparticle enzyme immunoassay (MEIA) for cTnI uses alkaline phosphatase conjugate. The cTnI assay using the Stratus analyzer also employs alkaline phosphatase. On the other hand, the chemiluminescent assay for troponin I does not use alkaline phosphatase. The concentration of alkaline phosphatase increases significantly in several diseases. Hepatobiliary diseases like obstructive jaundice, biliary cirrhosis, intrahepatic cholestasis, viral hepatitis, and cirrhosis can lead to significant increases in alkaline phosphatase concentrations. Several bone diseases and hyperparathyroidism also can lead to significant increase in alkaline phosphatase concentrations. Because a patient with suspected myocardial infarction may also have a clinical condition leading to elevated concentration of alkaline phosphatase, we studied the possible interference of alkaline phosphatase in three commonly used cTnI assays.

MATERIALS AND METHODS

We routinely receive specimens from patients with suspected myocardial infarction for the determination of troponin I concentrations. This study was performed using serum specimens after reporting of results ordered by the physicians. The microparticle enzyme immunoassay kits for cTnI were purchased from Abbott Laboratories (Abbott Park, IL), and the assay was run using an AxSYM analyzer. The chemiluminescent assay for cTnI was run using ACS:180 analyzer (Bayer Diagnostics, Tarrytown, NY). The fluorometric enzyme immunoassay kits for cTnI were purchased from Dade and the assay was run using a Stratus analyzer. Alkaline phosphatase enzyme in alkaline solution (pH 7.4) was purchased from Sigma Chemical Company (St. Louis, MO).

In the microparticle enzyme immunoassay (MEIA), cardiac troponin I in the patient's serum binds to the anti-troponin I microparticle, forming an antigen–antibody complex. Then the anti-troponin I:alkaline phosphatase conjugate is added to form antibody–antigen–antibody complex. Finally, 4-methylumbelliferyl phosphate is added, and the fluorescent product formed was measured in order to determine the concentration of troponin I in the specimen. The assay is linear up to a serum troponin I concentration of 50 ng/ml, and the sensitivity is 0.3 ng/ml. The minimum specimen requirement for the assay is 220 µl.

The chemiluminescent assay for troponin I is a competitive immunoassay where troponin I in the patient's sample competes with troponin I, which is covalently coupled to the paramagnetic particles in the solid phase for binding to the acridinium ester-labeled monoclonal troponin I antibody in the lite reagents. The assay is automated and requires 100 µl of specimen. The assay is linear up to a cTnI concentration of 50 ng/ml, and the sensitivity of the assay is 0.15 ng/ml.

The cTnI fluorometric enzyme immunoassay is an automated two-site immunoassay utilizing two monoclonal antibodies. The sample is pipetted onto the center of a square piece of glass fiber filter paper containing the capture antibody for cTnI. After a short incubation, another monoclonal antibody labeled with alkaline phosphatase is added to the reaction zone. After a second incubation, the excess antibody is washed off, a substrate for alkaline phosphatase is added, and the fluorescence is measured. The assay is linear up to a cTnI concentration of 50 ng/ml.

We added various amounts of a standard solution of alkaline phosphatase (ALP) to serum containing known concentration of cTnI, and then cTnI concentrations were measured again. The final ALP concentrations in sera were measured using a Hitachi 747 analyzer with the reagent pack supplied by the manufacturer following manufacturer's recommendations (Boehringer Mannheim, Indianapolis, IN).

Statistical analyses were done using an independent two-tailed *t*-test. A difference was considered statistically significant only at 95% confidence interval or higher ($P < 0.05$).

RESULTS AND DISCUSSION

We observed no interference of ALP in the MEIA cTnI assay. For example, in a serum pool containing 4.4 ng/ml of cTnI, the observed cTnI concentration even in the presence of 1,631 U/l of ALP was 4.6 ng/ml, a difference not statistically different from the control value. We did not observe any statistically significant change in other pools even in the presence of a ten times higher concentration than the upper end of normal ALP concentration. These results indicate that the MEIA assay for cTnI is completely free from the interference from ALP although ALP is used in the conjugate in this assay (Table 1).

As expected, the CLIA assay for cTnI is free from the interference from ALP because ALP is not used in this assay. In contrast, we observed significant interference of ALP in the fluorometric cTnI assay. The original specimen had a cTnI concentration of 0.5 ng/ml (ALP: 46 U/l). In the presence of 129 U/l of

TABLE 1. Effect of alkaline phosphatase on cTnI determination by MEIA

Specimen	ALP (U/l)[a]	cTnI (ng/ml), mean (SD), $n = 3$
Serum Pool 1	50	4.6 (0.35)
Serum Pool 1 + ALP	148	4.5 (0.14)
Serum Pool 1 + ALP	270	4.6 (0.10)
Serum Pool 1 + ALP	642	4.8 (0.07)
Serum Pool 1 + ALP	1,631	4.7 (0.49)
Serum Pool 2	65	20.9 (0.92)
Serum Pool 2 + ALP	198	20.7 (1.47)
Serum Pool 2 + ALP	291	20.5 (2.12
Serum Pool 2 + ALP	562	21.4 (1.56)
Serum Pool 2 + ALP	1,201	21.5 (0.87)

[a]ALP, alkaline phosphatase.

TABLE 2. Effect of ALP on cTnI measured by chemiluminescent (ACS:180) and fluorometric enzyme immunoassays (Stratus)

		CtnI (ng/ml), mean (SD), $n = 3$	
Specimen	ALP (U/l)	ACS:180	Stratus
Serum Pool 3	46	<0.15	0.5 (0.03)
Serum Pool 3 + ALP	129	<0.15	4.3 (0.10)[a]
Serum Pool 3 + ALP	222	<0.15	9.4 (1.12)[a]
Serum Pool 3 + ALP	190	<0.15	11.1 (0.87)[a]
Serum Pool 3 + ALP	445	<0.15	20.4 (2.12)[a]
Serum Pool 3 + ALP	913	<0.15	41.0 (1.27)[a]

[a]Significantly greater than the control value by the independent two-tailed *t*-test.

ALP, the observed cTnI concentration was falsely elevated to 4.3 ng/ml. Finally in the presence of 913 U/l of ALP, the observed cTnI concentration was 41.0 ng/ml (Table 2).

Butch et al. described interference of ALP in the CK-MB assay using the Stratus analyzer. In this sandwich assay, an ALP substrate washes unbound conjugate after the completion of immunoreaction and generates the fluorescent signal. High endogenous ALP present in the serum may not completely wash during the cycle, leading to falsely elevated results. The authors consulted with the manufacturer, and the successful reformulation of substrate/wash cycle minimized the interference (13). However, interference of ALP in the cTnI assay by the same manufacturer has not been reported before.

Recently, Altinier et al. evaluated rapid measurement of cardiac markers including cTnI using the Stratus analyzer. The authors concluded that the performance of cTnI assay using the Stratus analyzer has analytical reliability and satisfies the requirement specified in IFCC and the National Academy of Clinical Biochemistry for the measurement of cardiac markers. The authors further verified the stability of reagents, samples, and calibration curves as well as the on-board centrifugation capability (14).

Although cTnI is an excellent marker for the diagnosis of myocardial infarction, this is a relatively new test and like any other biochemical test is subject to interference. For example, Parry et al. recently reported discordance between the MEIA cTnI assay and CLIA cTnI assay and concluded that the MEIA assay is more prone to false-positive cTnI results than the CLIA assay for cTnI (15).

We conclude that the MEIA and CLIA cTnI assays are free from interference from high concentrations of ALP. However, the fluorometric enzyme immunoassay for cTnI using the Stratus analyzer is subjected to interference from high ALP concentrations.

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