Performance Evaluation of a New Chemiluminescent Cardiac Troponin I Assay

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Cardiac troponin I is a marker for diagnosis of myocardial damage. Several immunoassays are currently available for determination of concentrations of troponin I in serum. We evaluated a chemiluminescent assay for troponin I using ACS:180 automated analyzer (Bayer Diagnostics). We compared our results with two other immunoassays using the OPUS Magnum (OPUS troponin I assay, Dade Behring) and AxSYM (microparticle enzyme immunoassay, Abbott) analyzers. The within-run and between-run CVs were less than 5% for all three levels of controls. The chemiluminescent assay for troponin I was linear up to a serum troponin I concentration of 50 ng/mL and the detection limit was 0.1 ng/mL of troponin. A good correlation between troponin I concentration measured by the chemiluminescent assay (y axis) and the microparticle enzyme immunoassay (MEIA) (x axis) was observed, although the concentrations of troponin I in individual specimens were approximately four times higher, when measured by the MEIA assay, than those measured by chemiluminescent assay. The correlation coefficient was 0.98 with the regression equation $y = 0.22x + 1.125$. We also observed a good correlation in troponin I concentrations obtained by the chemiluminescent assay (y axis) and OPUS troponin I assay (x axis). The correlation coefficient was 0.96 and the regression equation was $y = 0.79x - 0.52$. The correlation coefficient was 0.93 when we compared troponin I concentrations obtained by the OPUS assay (x axis) with the corresponding concentrations obtained by the MEIA assay (y axis). The corresponding regression equation was $y = 0.25x + 3.5$. We conclude that the chemiluminescent troponin I assay showed good analytical performance. *J. Clin. Lab. Anal.* 14:224–229, 2000. © 2000 Wiley-Liss, Inc.

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Key words: troponin I; chemiluminescent; MEIA

INTRODUCTION

Cardiovascular disease is the major cause of morbidity and mortality in the United States. Atherosclerosis underlies virtually all cases of myocardial infarction. The precipitating event is often an acute occlusion of a coronary vessel from thrombosis of, 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 in diagnosis. Creatinine kinase isoenzyme (CK-MB) has been used for diagnosis of myocardial infarction for quite some time but this marker suffers from a number of serious problems (1,2). Concentrations of CK-MB can also be falsely elevated in alcoholics and after use of certain drugs like morphine (3,4). Elevated CK-MB concentrations in long-distance runners has also been reported.

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, play 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 and troponin I is considered a superior marker for diagnosis of myocardial infarction. Guest et al. published a report

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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. 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 an 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. This study, participating as one of the beta trial centers for Bayer Diagnostics, evaluated a new chemiluminescent assay for troponin I by comparing this new assay with two other widely used immunoassays.

MATERIALS AND METHODS

For the determination of troponin I concentrations, we routinely receive specimens from patients suspected to have myocardial infarction. This study was performed using leftover serum specimens after the reporting of results ordered by the physicians. The Institutional Review Board for the protection of human subjects approved this study. The specimens were analyzed using each analyzer within 4 hr. During that time specimens were kept at room temperature. Moreover, we did not observe any significant decline in troponin I concentration using these assays after up to 24 hr when specimens were stored overnight at 4°C.

The chemiluminescent assay for troponin I was run using the ACS:180 analyzer (Bayer Diagnostics, East Walpole, MA). This is a competitive immunoassay in which troponin I in a patient's sample competes with troponin I that is covalently coupled to the paramagnetic particles in the solid phase for binding to the acridinium ester labeled monoclonal troponin I antibody in the light reagents. The assay is automated and requires 100 µl of specimen. The microparticle enzyme immunoassay (MEIA) for troponin I was obtained from Abbott Laboratories and the assays were run using an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL). In this assay cardiac troponin I in the patient's serum binds to the anti-troponin I microparticle forming an antigen–antibody complex. Then anti-troponin I: alkaline phosphatase conjugate was added to form an antibody–antigen–antibody complex. Finally, 4-methylumbelliferyl phosphate was 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 was 220 µl. The OPUS troponin I assay (Dade Behring Diagnostics, Westwood, MA) is based on the principle of two-site or sandwich assay. The assay uses two goat polyclonal antibodies, which recognize different polypeptide segments unique to the cardiac isoform of

troponin I. Each test module contains one of these antibodies immobilized onto glass fiber. A solution of a second antibody conjugated to alkaline phosphatase and a wash/substrate solution containing 4-methylumbelliferyl phosphate is added later. The assays were performed on an OPUS Magnum analyzer. The calibration range of this assay is from 0 to 150 ng/mL of troponin I concentration. The sensitivity of the assay is 0.5 ng/mL.

RESULTS

Within-run and Between-run Precisions

The within-run and between-run precisions for the chemiluminescent assay for cardiac troponin I were determined by using Bio-Rad lipocheck cardiac controls. The within- and between-run precisions for the low control were 2.4% (mean = 3.71 ng/mL, SD = 0.09 ng/mL, $n = 10$), and 3.1% (mean = 3.86 ng/mL, SD = 0.12 ng/mL, $n = 10$), respectively. The within-run and between-run precisions for the medium controls were 3.0% (mean = 26.90 ng/mL, SD = 0.80 ng/mL, $n = 10$), and 3.8% (mean = 27.30 ng/mL, SD = 1.03 ng/mL, $n = 10$) respectively. Similarly, for the high control the within-run precision was 2.4% (mean = 47.10 ng/mL, SD = 1.11 ng/mL, $n = 10$) while the between-run precision was 3.3% (mean = 46.20 ng/mL, SD = 1.52 ng/mL, $n = 10$). In order to access precision at a very low level, we used another control (troponin I concentration: 0.66 ng/mL). The within-run and total CVs were 4.5 and 5.8%, respectively.

Linearity, Detection Limit, Dilution Recoveries, and Spiked Recoveries

The chemiluminescent assay for cardiac troponin I was linear up to a serum troponin I concentration of 50 ng/mL. This reportable range of linearity as stated by the manufacturer was further verified by adding a known amount of troponin I in specimens already containing troponin I. We observed expected increases throughout the reportable range. The detection limit was established by analyzing a zero calibrator for twenty times, then mean and standard deviations were calculated. The detection limit was set at 0.3 ng/mL, which was the value of mean + 2SD for the zero calibrator. The dilution recovery studies were performed by diluting different calibrators using dilution buffer. We used 1:2, 1:4, 1:8, and 1:16 dilutions. The percent recoveries varied from 82.9 to 106.7% with the mean recovery in each group varying from 93 to 102% (Table 1). Therefore, we conclude that the performance of the new cardiac troponin I assay was acceptable in the dilution recovery studies.

In a different set of experiments, we supplemented serum containing no measurable troponin I with various concentrations of troponin I, and compared the observed troponin I concentrations with the corresponding target troponin I concentration. The percent recoveries varied from 91.0 to

TABLE 1. Dilution recovery of the new chemiluminescent assay for cardiac troponin I

Experiment	Dilution	Cardiac troponin I (ng/mL)		Recovery (%)
		Observed	Expected	
1.	None	7.57		
	1:2	3.92	3.79	96.6
	1:4	1.96	1.89	96.8
	1:8	1.07	0.95	88.5
	1:16	0.54	0.47	88.0
2.	None	11.26		
	1:2	5.62	5.63	100.2
	1:4	3.05	2.82	92.2
	1:8	1.55	1.41	90.8
	1:16	0.80	0.70	88.0
3.	None	14.28		
	1:2	7.22	7.14	98.9
	1:4	3.67	3.57	97.3
	1:8	1.89	1.78	94.2
	1:16	0.97	0.89	91.7
4.	None	20.76		
	1:2	10.32	10.38	100.6
	1:4	5.33	5.19	97.3
	1:8	2.83	2.59	91.7
	1:16	1.56	1.30	82.9
5.	None	32.29		
	1:2	15.13	16.15	100
	1:4	7.84	8.07	102.9
	1:8	3.96	4.04	101.8
	1:16	2.08	2.02	97.0

110.9% (Table 2). Again we conclude that the new chemiluminescent troponin I assay's performance was acceptable in the spiked recovery studies.

Comparison of Methods

We measured the concentrations of troponin I in 50 specimens from 50 different patients using the MEIA, OPUS, and the new chemiluminescent assay. We observed a good correlation ($r = 0.98$) between the troponin I concentrations measured by the MEIA assay using the AxSYM analyzer (x axis) and the new chemiluminescent assay (y axis), using the ACS:180 analyzer. However, the concentration of troponin I in individual specimens was roughly four times higher when measured by the MEIA assay compared to the concentrations measured by the chemiluminescent assay. The regression equation was

$$y = 0.22x + 1.125 \text{ (Fig. 1).}$$

The Sy/x value was 6.54.

We also used another regression analysis using data in which troponin I concentrations were less than 10 ng/mL. Using the x axis to show the troponin I concentration observed by the MEIA assay performed by an AxSYM analyzer, and using the y axis to show the chemiluminescent assay performed by an ACS:180 analyzer, we observed the regression equation

TABLE 2. Spiked recovery of the new chemiluminescent assay for cardiac troponin I

Experiment	Spiked dose (ng/mL)	Cardiac troponin I (ng/mL)		Recovery (%)
		Observed	Expected	
1.	Serum pool 1	0.05		
	2.0	2.10	2.05	102.4
	5.0	5.14	5.05	101.9
	20.0	19.11	20.05	95.3
	40.0	36.53	40.05	91.2
2.	Serum pool 2	0.78		
	2.0	2.63	2.78	94.7
	5.0	5.78	5.78	100.0
	20.0	19.36	20.78	93.2
	40.0	39.86	40.78	97.7
3.	Serum pool 3	6.49		
	2.0	9.42	8.49	110.9
	5.0	12.55	11.49	109.2
	20.0	26.44	26.49	99.8
	40.0	44.12	46.49	94.9
4.	Serum pool 4	10.34		
	2.0	11.98	12.34	97.1
	5.0	14.13	15.34	92.1
	20.0	27.60	30.34	91.0
	40.0	45.92	50.34	91.2

$$y = 0.25x + 0.06 \text{ (} r = 0.97, n = 30 \text{) (Fig. 2).}$$

The Sy/x value was 0.17.

We also observed a good correlation (0.96) between the troponin I concentrations measured by the OPUS assay (x axis) using the Magnum analyzer and the chemiluminescent assay (y axis) using the ACS:180 analyzer. In this case the troponin I concentration in individual specimens measured by the chemiluminescent assay was slightly higher (approximately 20%) than when measured by the OPUS assay. The regression equation was

$$y = 0.79x - 0.52 \text{ (Fig. 3).}$$

The Sy/x value was 8.73.

We also used another regression analysis using data where troponin I concentrations were less than 6 ng/mL. Using the x axis to show the troponin I concentration observed using the Magnum analyzer and using the y axis to show the values observed by using the ACS:180 analyzer, we observed the following regression equation

$$y = 0.82x - 0.23 \text{ (} r = 0.94, n = 37 \text{) (Fig. 4).}$$

The Sy/x value was 0.51.

The correlation coefficient was 0.93 when we compared the troponin I concentrations obtained by the MEIA assay (x axis) to those of the OPUS assay (y axis). Interestingly, for individual specimens, the concentration of troponin I measured by the MEIA assay was more than four times higher

Fig. 1. Regression analysis using the *x* axis to show the cardiac troponin I concentrations observed by the MEIA (AxSym) assay and the *y* axis to show

the cardiac troponin I concentrations observed by the chemiluminescent assay (ACS:180).

than the corresponding troponin I concentration measured by the OPUS assay.

The regression equation was

$$y = 0.25x + 3.5.$$

The *Sy/x* value was 14.21.

We also used another regression analysis using data in which troponin I concentrations were less than 10 ng/mL.

With the *x* axis showing the troponin I concentration observed by using the AxSYM analyzer, and *y* axis showing the values observed using an ACS:180 analyzer, we observed the regression equation

$$y = 0.28x + 0.39 \text{ (} r = 0.85, n = 30 \text{) (Fig. 4).}$$

The *Sy/x* value was 0.34.

Fig. 2. Regression analysis using the *x* axis to show the cardiac troponin I concentrations observed by the MEIA (AxSym) assay and the *y* axis to show the cardiac troponin I concentrations observed by the new chemiluminescent assay (ACS:180). The values of cardiac troponin I used in this regression plot were less than 10 ng/mL.

DISCUSSION

Various clinical studies have demonstrated the release of cardiac troponin I into the blood stream within hours following myocardial infarction or ischemic damage. Elevated concentrations of troponin I are detectable in serum within 4 to 6 hours after the onset of chest pain. The peak concentrations may be reached within 12 hours and the concentrations may stay elevated for 3 to 10 days. The pattern of troponin I release following myocardial infarction thus extends across the diagnostics windows of both CK-MB and lactate dehydrogenase (13). The clinical utility of troponin I for evaluating myocardial infarction in patients has been clearly demonstrated in the literature. Moreover, troponin I also has improved cardiac specificity over the conventional marker, CK-MB.

Different immunoassays report different values and different guidelines should be followed for interpretation of results. For example, the guidelines from Abbott Laboratories

Fig. 3. Regression analysis using the x axis to show the cardiac troponin I concentrations observed by the FIA (Magnum) and the y axis to show the

cardiac troponin I concentrations observed by the new chemiluminescent assay (ACS:180).

suggest that the diagnostic cutoff for myocardial infarction is 2.0 ng/mL of serum troponin I concentration. Healthy individuals should have a troponin I concentration below 0.4 ng/mL (package insert, Abbott Laboratories). On the other hand

Fig. 4. Regression analysis using the x axis to show the cardiac troponin I concentrations observed by the FIA (Magnum) and the y axis to show the cardiac troponin I concentrations observed by the new chemiluminescent assay (ACS:180). The values of cardiac troponin I used in this regression plot were less than 6 ng/mL.

Behring Diagnostics, the manufacturer of the OPUS troponin I assay, suggests that any troponin I concentration over 0.5 ng/mL may indicate a myocardial infarction. This is expected because the troponin I concentration measured by the MEIA assay is usually four times higher than the corresponding troponin I concentration measured by the OPUS assay. The troponin I concentrations measured by the chemiluminescent assay were closer to the concentrations measured by the OPUS assay (about 15–20% difference).

The main reason for this discrepancy is the lack of standardization in troponin I. Troponin I exists in various isoforms, free, and in complex form. The complex can be binary (troponin I + troponin C) or ternary (troponin I + troponin C + troponin T). Additionally there are the oxidized form and the sulfate conjugation of troponin. Most troponin forms are liable and pure preparations are not easily available. Moreover, no international reference material of troponin is available. Different antibody combinations used in various commercial kits widely vary in epitope recognition and specificity to the isoforms. Some kits use two antibodies whereas the chemiluminescent assay uses three antibodies: two monoclonals on solid phase (capture) and one polyclonal on the light reagent (label).

Therefore, the differences in absolute troponin I concentrations measured by different immunoassays are expected.

More importantly, the values should be consistent with the clinical picture. When a troponin I concentration was above the reference range by the chemiluminescent assay, the corresponding troponin concentrations measured by the OPUS and MEIA assays were also above the corresponding reference ranges of these assays. Although the regression equation exhibited significant difference between the troponin values measured by the chemiluminescent assay and the MEIA assay, the excellent correlation coefficient assures that the chemiluminescent assay is equally effective in diagnosis of myocardial infarction as the MEIA assay. Moreover, the already FDA-approved OPUS assay also showed similar bias with the MEIA assay.

We conclude that the new chemiluminescent assay for troponin I has acceptable analytical performance and can easily be adopted into a clinical laboratory for the routine measurement of troponin I.

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