Journal of Clinical Laboratory Analysis 15:76–80 (2001)

Negative Interference of Bilirubin and Hemoglobin in the MEIA Troponin I Assay But Not in the MEIA CK-MB Assay

Amitava Dasgupta,* Alice Wells, and David A. Biddle

Department of Pathology and Laboratory Medicine, University of Texas–Houston Medical School, Houston, Texas

Troponin I is a sensitive and specific marker for the diagnosis of myocardial infarction. Several commercially available immunoassays measure the concentration of troponin I in serum. The microparticle enzyme immunoassay (MEIA) for troponin I (Abbott Laboratories, Abbott Park, IL) is widely used in clinical laboratories, including our hospital laboratory. We studied the effect of bilirubin and hemolysis on the MEIA for troponin I and compared our assay with a newly available chemiluminescent assay (CLIA) for troponin I (Bayer Diagnostics, Tarrytown, NY). We also measured CK-MB concentration using the MEIA CK-MB assay. One serum pool was prepared by combining several specimens of one patient with elevated troponin I and with a diagnosis of myocardial infarction. Other serum pools were prepared by combining sera with similar troponin I values. All serum pools showed normal bilirubin concentrations and had no hemolysis. Then we supplemented aliquots of serum pools with various concentrations of bilirubin (5.0, 10.0, 15.0, and 20.0 mg/dL). After supplementation, troponin I concentrations were measured again using the MEIA and CLIA. We observed a statistically significant decrease in troponin I concentration in the presence of bilirubin with the MEIA. For example, in serum pool 1, the troponin I concentration was 16.3 (bilirubin: 0.8 mg/dL). In the presence of 5.0, 10.0, 15.0 and 20.0 mg/dL of added bilirubin, the cardiac troponin I concentrations were 13.9, 13.4, 13.3 and 13.0 ng/ml respectively. We observed similar negative interference of bilirubin in troponin I measurement by the MEIA in other pools. The troponin I value decreased slightly (not statistically significant) in one pool and did not change in two other pools in the presence of bilirubin when we measured troponin I concentration using the CLIA. Interestingly, bilirubin did not interfere with the MEIA CK-MB assay. Moderate hemolysis did not have any effect on the troponin I assay using either the MEIA or CLIA. However, gross hemolysis (hemoglobin > 40 mg/dL) interfered with both assays for troponin I. J. Clin. Lab. Anal. 15:76–80, 2001.

Key words: troponin I; microparticle enzyme immunoassay; bilirubin; hemolysis

INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality in the U.S. Atherosclerosis underlies virtually all cases of myocardial infarction. The dying myocardial cells release their content into the cardiac lymphatic and the bloodstream. Therefore, increased concentrations of cardiac enzymes such as creatinine kinase and lactate dehydrogenase can be detected in the blood after an episode of myocardial infarction. Creatinine kinase isoenzyme (CK-MB) has been used as a marker for myocardial infarction, but the marker has certain limitations (1,2). Troponin I is the regulatory subunit of the troponin I complex associated with the actin thin filament within muscle cells. Guest et al. published a report on 209 complex patients to determine the incidence of effect of unrecognized cardiac injury in critically ill patients and concluded that troponin I is a superior marker for the diagnosis of myocardial injury (3). Troponin I is very specific because it is found only in the heart muscle. Other publications also indicate that troponin I concentration is not falsely elevated in hypothyroidism, whereas the CK-MB concentration may be falsely elevated (4). Moreover, uremia and skeletal muscle trauma do not lead to an increase in troponin I concentration whereas the CK-MB fraction again may be falsely elevated (5–8).

Several commercially available immunoassays measure the concentration of troponin I in serum. The microparticle enzyme immunoassay (MEIA) for troponin I, marketed by Abbott Laboratories, is widely used in clinical laboratories including our hospital laboratory. Recently, ver Elst et al.

*Correspondence to: Dr. Amitava Dasgupta, Department of Pathology and Laboratory Medicine, University of Texas–Houston Medical School, 6431 Fannin, MSB 2.292, Houston, TX 77030.

Received 10 August 2000; Accepted 9 October 2000

evaluated the MEIA assay of troponin I and observed negative interference of bilirubin and hemoglobin in the assay (9). The authors used two serum pools to evaluate the interference of bilirubin. In one pool, the authors observed a 100% decrease in troponin I concentration in the presence of 10 mg/dL of bilirubin and in another specimen observed a less significant—15.3%—decline in the presence of 20 mg/dL of bilirubin. Bock, in his recent editorial, commented that the troponin I assay is subject to various interferences and results should be interpreted carefully (10).

Because the MEIA troponin I assay is widely used in clinical laboratories, we investigated in detail the interference of bilirubin and hemolysis in this assay using low, moderate, and high concentrations of bilirubin and hemoglobin. We also compared our result with a newly available chemiluminescent assay for troponin I marketed by Bayer Diagnostics. In our previous study, we showed that although the MEIA troponin I assay was subject to interference from rheumatoid factors, the new chemiluminescent assay was free from this interference (11). We also measured CK-MB concentrations in order to study the interference of bilirubin and hemolysis, because CK-MB values are widely used by cardiologists for diagnosis.

MATERIALS AND METHODS

The MEIA assay kits for troponin I were purchased from Abbott Laboratories (Abbott Park, IL) and the new chemiluminescent immunoassay (CLIA) troponin I kits were obtained from Bayer Diagnostics (Bayer, Tarrytown, NY). The MEIA assay for troponin I was run on an AxSYM analyzer and was also obtained from the Abbott Laboratories. The CLIA assay was run on an ACS:180 analyzer obtained from Bayer Diagnostics. Bilirubin (mixed isomers) and hemoglobin were purchased from Sigma Chemical Company (St. Louis, MO). Bilirubin concentrations in sera were measured using a Hitachi 747 analyzer. CK-MB concentrations were measured using the MEIA and AxSYM analyzer (Abbott Laboratories).

We prepared serum pools from sera of patients with various concentrations of troponin I. Such specimens are routinely submitted by clinicians and the study was done using the leftover specimens, which would otherwise have been discarded, after reporting results to the ordering physicians. The use of left over specimens for research without identifying individual patient and/or reviewing patient's chart is approved by the IRB. The serum pool 1 was prepared by combining several specimen of one patient with a positive diagnosis of myocardial infarction. Other serum pools were prepared by combining specimens with similar troponin I concentrations. This approach to the preparation of serum pools for investigating interference of bilirubin and hemolysis on troponin I assay is similar to that of ver Elst et al. (9). We also compared troponin I concentrations measured by the MEIA and CLIA in individual specimens containing various concentrations of bilirubin and also in hemolyzed specimens. These patients had a variety of clinical diagnoses including myocardial infarction, unstable angina, and critical illness.

A stock solution of bilirubin (10 mg/ml) was prepared in 0.1 N sodium hydroxide. Then, to 2-ml aliquots of serum pool, 10, 20, 30, or 40 μl of stock bilirubin solution was added. The final expected bilirubin concentrations were 5, 10, 15, and 20 mg/dL respectively. Troponin I concentrations were measured by both assays after supplementation with bilirubin. The dilutions of specimens were minimal (0.5–2.0%). Therefore, in interpreting results dilution factors were ignored. Bilirubin concentrations in these specimens were also measured to verify correct supplementation using bilirubin stock solution.

In another experiment, microliter amounts of stock hemoglobin solution were added to aliquots of serum pool to mimic hemolysis. Troponin I concentrations were measured by both assays after supplementation with hemoglobin. Again dilution factors were ignored in interpreting results.

We also measured cardiac troponin I concentrations using both MEIA and CLIA in eight individual specimens. Three specimens were moderately hemolyzed and another five specimens had upper-end-of-normal to elevated bilirubin concentrations. No supplementation of bilirubin or hemoglobin was done to these specimens.

Statistical analyses were performed using the independent *t*-test, two tailed. We considered a difference statistically significant only at a 95% confidence interval or higher ($P < 0.05$).

RESULTS

We observed statistically significant decreases in troponin I values in the presence of bilirubin using the MEIA assay. Our results are in agreement with initial observations made by ver Elst et al. (9). However, the magnitude of interference was less in our study. We observed a statistically significant decline in cardiac troponin I concentration even when an aliquot of a serum pool was supplemented with only 5.0 mg/dL of bilirubin. For example, in serum pool 1, the troponin I concentration was 16.3 ng/ml (bilirubin: 0.8 mg/dL). In the presence of 5.0, 10.0, 15.0, and 20.0 mg/dL of added bilirubin, the troponin I concentrations were 13.9, 13.4, 13.3, and 13.0 ng/ml respectively (Table 1). Interestingly, we did not observe any statistically significant decline or increase in the CK-MB concentrations in the presence of bilirubin when concentrations were measured by the MEIA CK-MB assay. We observed a slight but statistically insignificant decline in cardiac troponin I concentration in the presence of 20 mg/dL of added bilirubin in one serum pool using the CLIA assay. However in other serum pools, no change in value in the presence of bilirubin was observed using the CLIA assay (Table 1).

We observed a slight but statistically insignificant decline in troponin I concentrations in the presence of moderate con-

TABLE 1. Effect of bilirubin on the MEIA troponin I assay, CLIA troponin I assay, and MEIA CK-MB assay

	Bilirubin mg/dL		Troponin I, ng/ml		
			MEIA	CLIA	CK-MB U/L (MEIA)
Specimen ID	Added	Observed	Mean (SD), n = 3		Mean (SD), n = 3
Serum pool 1 (control)	0.0	0.8	16.3 (0.8)	3.2 (0.2)	15.1 (0.4)
Serum pool 1	5.0	6.0	13.9 (0.2)[a]	3.1 (0.1)	15.3 (1.5)
Serum pool 1	10.0	10.4	13.4 (0.1)[a]	3.1 (0.1)	15.2 (0.3)
Serum pool 1	15.0	14.8	13.3 (0.2)[a]	3.0 (0.2)	14.8 (0.1)
Serum pool 1	20.0	20.2	13.0 (0.2)[a]	2.9 (0.1)	14.6 (1.1)
Serum pool 2 (control)	0.0	0.2	4.1 (0.1)	0.95 (0.1)	10.2 (0.3)
Serum pool 2	5.0	5.8	3.8 (0.1)[a]	0.95 (0.1)	10.1 (0.1)
Serum pool 2	10.0	11.0	3.6 (0.2)[a]	0.95 (0.1)	9.9 (0.2)
Serum pool 2	15.0	16.8	3.4 (0.3)[a]	0.93 (0.2)	9.7 (0.3)
Serum pool 2	20.0	21.8	3.4 (0.2)[a]	0.94 (0.3)	10.0 (0.1)
Serum pool 3	0.0	0.4	2.6 (0.1)	0.39 (0.1)	6.3 (0.1)
Serum pool 3	5.0	6.0	2.3 (0.1)[a]	0.39 (0.1)	6.2 (0.1)
Serum pool 3	10.0	12.3	2.2 (0.1)[a]	0.37 (0.1)	5.9 (0.3)
Serum pool 3	15.0	15.8	2.1 (0.2)[a]	0.39 (0.1)	6.0 (0.2)
Serum pool 3	20.0	19.7	2.0 (0.1)[a]	0.40 (0.1)	6.0 (0.2)

[a]Significantly less than the corresponding control troponin I concentration by the independent *t*-test, two tailed ($P < 0.05$).

centrations of hemoglobin (20 mg/dl) when troponin I concentrations were measured by the MEIA. However, with gross hemolysis (hemoglobin: 40 mg/dl), we observed a statistically significant decline in troponin I concentrations measured by the MEIA (Fig. 1). Gross hemolysis also interfered with the CLIA cardiac troponin I assay.

We observed good correlation between troponin I concentrations measured by the MEIA and CLIA in specimens with normal to moderately elevated bilirubin concentrations. We also observed good correlation in troponin I values in mild to moderately hemolyzed specimens analyzed by both MEIA and CLIA. However, in two specimens with high bilirubin, the correlation between the troponin I values was poor. The MEIA assay underestimated the cardiac troponin I concentration (Table 2).

DISCUSSION

The decline in concentration of troponin I in the presence of 20 mg/dL of bilirubin was approximately 20% in the MEIA assay for troponin I in all three serum pools we studied. Although our observation of negative interference of bilirubin in the MEIA troponin I assay was similar to the observation reported by ver Elst et al. (9), the magnitude of bilirubin interference was much smaller in our study. Ver Elst et al. studied bilirubin interference using two serum pools. In one serum pool the concentration of troponin I decreased from 1.3 to 0.4 ng/ml in the presence of 10.0 mg/dL of bilirubin (a 69.2% decrease) and finally to 0.0 ng/ml in the presence of 20.0 mg/dL of bilirubin (a 100% decrease). In the second serum pool, the authors observed a decline of troponin I value from 29.4 to 24.9 ng/ml in the presence of 20 mg/dl of bilirubin (only a 15.3% decrease). Therefore, there is a discrepancy in the extent of interference of bilirubin in the two pools. However, in our study, we observed a 20.2% decline in troponin I concentration in the presence of 20 mg/dL of bilirubin in pool 1, a 17.1% decline in pool 2, and a 23.0% decline in pool 3 (Table 1). The magnitude of interference of bilirubin is comparable in all three pools we studied.

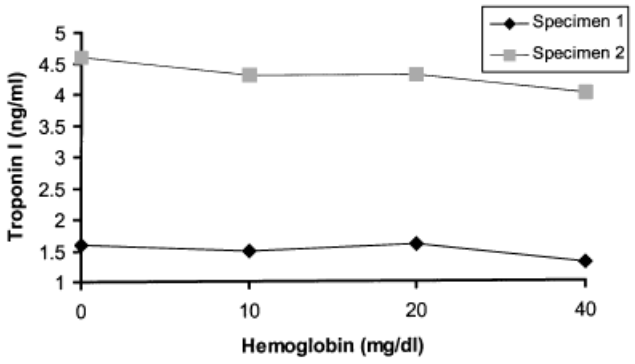

Fig. 1. Effect of added hemoglobin on cardiac troponin I values measured by the MEIA.

The decline in cardiac troponin I concentration in the presence of bilirubin is not related to the matrix. We dissolved bilirubin mixed isomers in sodium hydroxide and added only microliter quantities of standard solution in milliliter quantities of blood. Glick et al. studied interference of bilirubin in various laboratory tests. The authors used dimethyl sulfoxide and sodium carbonate solution to prepare stock solution for their studies (12). We used only alkaline solution to prepare stock solution in order to avoid any matrix effect. The measured bilirubin concentrations were in agreement with expected bilirubin concentrations indicating that there was no matrix effect following addition of bilirubin stock solution to serum pools. Moreover, we used the same approach to study the potential interference of bilirubin in the CK-MB assay using the same technology and observed no significant inter-

TABLE 2. Troponin I concentrations in eight individual specimens (bilirubin or hemolysis) measured by the MEIA and CLIA

		Troponin I concentration, ng/ml		
Specimen ID	Bilirubin/dL	MEIA (observed)	CLIA (calculated)[a]	CLIA (observed)
A	13.5	2.3	0.6	1.3
B	14.8	6.0	1.6	3.1
C	3.5	4.8	1.3	1.1
D	3.2	7.5	1.9	1.7
E	0.8	7.3	1.9	1.9
F	Hemolysis (2+)	4.4	1.2	1.2
G	Hemolysis (1+)	94.3	21.9	20.9
H	Hemolysis (2+)	6.2	1.6	1.7

[a]CLIA (calculated) was obtained by using the observed MEIA value and the regression equations described in the text.

ference. Therefore, we conclude that observed negative interference of bilirubin in the MEIA cardiac troponin I assay is not related to matrix.

Although the decline in concentration of cardiac troponin I as measured by the MEIA was statistically significant even in the presence of 5 mg/dL of bilirubin, the declines may not be clinically significant especially for moderate to elevated troponin I concentrations. Troponin I concentrations may increase 5- to 50-fold of the upper end of normal range in patients with myocardial infarction (13,14). Cina et al. reported a mean troponin I level of 93.4 ng/ml in cardiac related death (15). Therefore, low to moderate underestimation of troponin I values by the MEIA may not have any effect on the final outcome of diagnosis of the patient. Moreover, CK-MB values measured by the same MEIA technology and the same AxSYM analyzer are not subject to interference from elevated concentrations of bilirubin. Most clinicians in our hospital order both troponin I and CK-MB and compare both results for the evaluation of patients.

The CLIA assay for troponin I is virtually free from bilirubin interference. This assay uses a chemiluminescent technique and fluorescence of bilirubin can not interfere. On the other hand, the MEIA assay uses the fluorescence polarization technology.

We described earlier a good correlation between troponin I concentrations measured by the MEIA and CLIA (16), although the individual values measured by the MEIA were roughly four times higher than the values measured by the CLIA. Using the *x* axis as the troponin I concentrations measured by the MEIA and the *y* axis as the troponin I concentrations measured by the CLIA, we observed the following regression equation for troponin I concentrations less than 10 ng/ml (by the MEIA):

$$y = 0.25\ x + 0.06.$$

For troponin I concentrations over 10 ng/ml as measured by the MEIA, the regression equation was:

$$y = 0.22 + 1.125$$

We used these regression equations to estimate troponin I values by the CLIA using the observed troponin I with the MEIA in specimens with normal, moderately elevated, and elevated bilirubin. No supplementation of bilirubin was done. As expected, we observed a good correlation between the expected value and the observed CLIA value in specimens with normal to moderately elevated bilirubin concentrations. However, in two specimens with high bilirubin concentrations, the estimated values were lower than the observed values (CLIA), indicating that the MEIA underestimated troponin I concentrations in these specimens containing high bilirubin concentrations (Table 2).

Interestingly, moderate hemolysis (2+) had no significant effect on either troponin I assay. Moreover, Abbott's CK-MB assay also did not show any significant interference with mild to moderate hemolysis. Therefore, mild to moderately hemolyzed specimens can be accepted by the laboratory for troponin I or CK-MB assay. However, gross hemolysis interfered with both assays and grossly hemolyzed specimens should be rejected.

We conclude that both bilirubin and hemolysis had negative interference with the MEIA troponin I assay. However, mild to moderate hemolysis should not significantly alter troponin I results. For significantly elevated troponin I values, negative interference of bilirubin may not alter the clinical outcome. However, for values around the cut-off of 2.0 ng/ml, caution should be exercised in interpreting troponin I results if MEIA is used. The CLIA is free from interference from bilirubin. The MEIA CK-MB assay was also free from interference of bilirubin.

REFERENCES

1. Dorogy ME, Hooks GS, Cameron RW, Davis RC. Clinical and angiographic correlates of normal creatinine kinase with increased MB isoenzymes in possible acute myocardial infarction. Am Heart J 1995;130:211–217.
2. Hong RA, Litch JD, Wei Y, et al. Elevated CK-MB with normal total creatinine kinase in suspected myocardial infarction: associated clinical findings and early prognosis. Am Heart J 1986;111:1041–1046.
3. Guest TG, Ramanathan AV, Tuteur PG, et al. Myocardial injury in critically ill patients; a frequent unrecognized complication. JAMA 1995;273:1945–1949.
4. Cohen LF, Mohabeer AJ, Keffer JH, Jialal I. Troponin I in hypothyroidism. Clin Chem 1996;42:1494–1495.

5. Beck SR. Myocardial injury in critically ill patients. JAMA 1995[letter];274:1672–1673.
6. Li D, Jialal I, Keffer JH.Greater frequency of increased cardiac troponin I than increased cardiac troponin T in patients with chronic renal failure. Clin Chem 1996;42:114–115.
7. Keffer JH. The cardiac profile and proposed practice guideline for acute ischemic heart disease. Am J Clin Pathol 1997;107:398–409.
8. Bodor GS, Porterfield D, Voss EM, Smith S, Apple FS. Cardiac troponin I is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue. Clin Chem 1995;41:1710–1715.
9. ver Elst KM, Chapelle JP, Boland P, Demolder JS, Gorus FK. Analytical and clinical evaluation of the Abbott AxSYM cardiac troponin I assay. Am J Clin Pathol 1999;112:745–752.
10. Bock JL. Cardiac troponin . How specific is specific? Am J Clin pathol 1999;112:739–741.
11. Dasgupta A, Banerjee S, Datta P. False positive troponin I in the MEIA due to the presence of rheumatoid factors in serum: elimination of this interference by using a polyclonal antisera against rheumatoid factors. Am J Clin Pathol 1999;112:753–756.
12. Glick MR, Ryder K, Jackson S. Graphical comparisons of interferences in clinical chemistry instrumentation. Clin Chem 1986;32:470–475.
13. Adams JE, Bodor GS, Davilla-Roman, et al. Cardiac troponin I: a marker with high specificity for cardiac injury. Circulation 1993;88:101–106.
14. D'Costa M, Fleming E, Patterson MC. Cardiac troponin I for the diagnosis of acute myocardial infarction in emergency department. Am J Clin Pathol 1997;108:550–555.
15. Cina SJ, Li DJ, Chan DW, et al. Serum concentrations of cardiac troponin I in sudden death: a pilot study. Am J Forensic Med 1998;19: 324–328.
16. Dasgupta A, Chow L, Nazareno L, Tso G, Datta P. Performance evaluation of a new chemiluminescent cardiac troponin I assay. J Clin Lab Anal 2000;14:224–229.