Positive Interference from Contrast Media in Cardiac Troponin I Immunoassays

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Cardiac troponin I (cTnI) has been found to be a sensitive and reliable marker of myocardial damage, and elevated levels of cTnI can indicate high risk for acute coronary syndrome. To determine how to intervene in possible cases of acute coronary syndrome, cTnI levels must be measured by immunoassay. However, cTnI immunoassay results are prone to interference from many substances such as heparin and common drugs. The contrast media used in the coronary angiography might also interfere with results. To explore this possibility, we performed two *in vivo* and two *in vitro* studies. In the first *in vivo* study, we evaluated the effects of contrast media on cTnI immunoassays by collecting blood samples from 45 patients undergoing coronary angiography before and after the procedure. We used the Opus Magnum immunoassay system to measure cTnI levels. In the second *in vivo* study, we collected 25 blood samples from another group of patients also undergoing angiography at various times before and after the procedure to determine cTnI values by both the Opus Magnum and ACCESS systems. In the first *in vitro* study, 12 different contrast media were treated as samples to disclose the potential interference of measurement in the two assay systems. In the second *in vitro* study, we made sequential dilutions of iopromide (Ultravist; Schering) with serum to explore their potential for interfering with the detection of cTnI by the Opus Magnum and ACCESS assays. In the first *in vivo* study using the Opus Magnum assay, cTnI concentrations in samples taken after angiography were significantly higher at 5 minutes than at 30 minutes, and, at 60 minutes, all cTnI concentrations had dropped below the cutoff point. In the second *in vivo* study, we found a substantial difference in detection of cTnI by the Opus Magnum and ACCESS assays. All cTnI concentrations checked by ACCESS assay were below the cutoff value. In our first *in vitro* study, the Opus Magnum assay gave false positive results for all 12 contrast media; the ACCESS assay gave a positive result for only one contrast medium, poppy-seed oil (Lipiodol; Guebert). In our second *in vitro* study, we found that, in the Opus Magnum assay, the more concentrated the contrast medium, the higher the cTnI value, but not in the ACCESS assay. We conclude that contrast media may cause false-positive results in cTnI assays and that, when contrast media are being used for angiography, cTnI results, especially those based on samples taken within the first hour of the procedure, should be interpreted carefully.

Key Words: immunoassay, interference, cardiac troponin I, contrast media
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Cardiac troponin I (cTnI) is a sensitive and reliable marker of myocardial damage, and it can be used to diagnose acute coronary syndrome [1,2]. Because therapeutic decisions may be made based on relatively small increases in cTnI, troponin assays must have high sensitivity and specificity.

The molecular complexity of cTnI causes discrepancies in its measurement by different assays [3]. In addition,

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because the immunoassays measuring cTnI use an array of various antibodies, the assays are prone to interference, which has been reported to occur with the use of heparin, blood constituents, hemolysis, bilirubin, common drugs, heterophilic antibodies, human anti-mouse antibodies, and rheumatoid factors [4–6].

Contrast media are essential for all radiologic angiographies, including coronary angiography, and are also essential for percutaneous coronary intervention. Although cardiac damage can be practically and sensitively detected after percutaneous coronary intervention by measuring cTnI, few clinicians know that commercial assays of troponin are prone to interference [4–6]. Because it was not known whether contrast media can interfere with immunoassay for cTnI, we used two *in vivo* and two *in vitro* studies to observe the effects of various contrast media on the results of two cTnI immunoassays: the Opus Magnum immunoassay and the ACCESS immunoassay.

Materials and Methods

cTnI Measurement

cTnI concentrations were measured using two automatic commercial assays: Opus Magnum (Opus cTnI immunoassay system; Behring Diagnostics) and ACCESS cTnI immunoassay; Beckman Coulter, Inc.). The manufacturer-recommended cutoffs for myocardial injury were set at 0.5 ng/mL for Opus Magnum and 0.15 ng/mL for ACCESS. The range of testing was 0.5–150 ng/mL for Opus Magnum and 0–30.0 ng/mL for ACCESS.

In vivo *studies*

Study population

We did two *in vivo* studies. In the first study, we tested whether the coronary angiography and angioplasty had caused damage to the myocardium by checking the cTnI level on the Opus Magnum system before and after the procedure. We found unusually high cTnI concentrations. To confirm whether the contrast media had positively interfered with the cTnI immunoassay results, in the second *in vivo* study, we used both the Opus Magnum and ACCESS systems.

In the first *in vivo* study, we enrolled 45 patients scheduled to receive coronary angiography with or without coronary intervention. Of these patients, 14 received coronary intervention. Eighty percent of the 45 patients were men. The mean age was 57.9 years (range 22–75 years). Patients with suspected acute coronary syndrome or renal function impairment (serum creatinine > 1.5 mg/dL) were excluded. In the second *in vivo* study, we enrolled 25 patients. Inclusion and exclusion criteria were the same. Informed consent forms were signed by all the participants.

Coronary angiography and blood sample collection

The contrast medium used for the coronary angiography was iopromide (Ultravist; Schering). In the first *in vivo* study, 5 mL of blood was collected in clot activator tubes (serum separation tubes) immediately before the procedure and at 5 minutes, 30 minutes, 60 minutes, and 6 hours after the procedure. In the second *in vivo* study, blood samples were collected immediately before, and at 5 minutes and 30 minutes after the procedure. All blood samples were drawn from the sidearm of the femoral arterial sheath. Approximately 20 minutes after being collected, specimens were centrifuged at 3000 *g* (KUBOTA 2700) for 5 minutes. The serum was then immediately analyzed or frozen and thawed just before analysis. For the Opus Magnum immunoassay, 40 μL of serum was used and, for the ACCESS assay, 50 μL. All cTnI concentrations were tested in duplicate.

In vitro *studies*

The first *in vitro* study was done to observe whether different contrast media could positively interfere with cTnI immunoassay results. For both immunoassays, 12 different contrast media were used as substrates. For each test the specimen amount was 50 μL for the ACCESS assay and 40 μL for the Opus Magnum assay. The contrast media we tested were iothalamate (Conray60; Mallinckrodt), ioversol (Optiray320; Mallinckrodt), iohexol (Omnipaque300/350; NycoMed), diatrizoate sodium (Hypaque76; Sanofi Winthrop), diatrizoate methylglutamine (Angiografin60; Schering), gadopenetic acid (Magnevist; Schering), megluminamidotizoate (Urografin76; Schering), iopromide (Ultravist300/370; Schering), iodipamide (Biligrafin; Schering), and iodized ethylesters of poppy-seed oil (Lipiodol; Guebert). In addition, one sample of serum from a patient with acute myocardial infarction (AMI) was used as the positive control. All tests were performed in triplicate.

The second *in vitro* study was done to measure the effect of contrast media concentration on cTnI immunoassay results. This was done by mixing 0, 0.1, 0.2, 0.3, or 0.4 mL of Ultravist370 with 5 mL of whole blood from the same patient. The mixtures were centrifuged at 3000 *g* for 5 minutes, and then 40 μL of serum was pipetted for each Opus Magnum assay and 50 μL of serum for each ACCESS assay. One specimen from a patient with AMI was used as

the positive control. All tests were performed in triplicate. The reported cTnI concentration was the average of the three tests.

Statistics

The paired Student's *t*-test was used to determine the difference between cTnI values of samples taken at different times. The difference between the Opus Magnum and ACCESS immunoassay systems was analyzed by unpaired *t*-test. Wilcoxon signed-rank test was used to determine the difference in the cTnI values collected from patients undergoing coronary angiography and percutaneous coronary intervention. A two-tailed $p < 0.05$ was considered statistically significant. All analyses were done on SPSS 11.

Results

In the first *in vivo* study, the baseline cTnI concentration was lower than the cutoff value in all of the 45 patients before the angiography. At 5 minutes after the procedure, this value was found by Opus Magnum assay to be elevated (mean 19.68 ng/mL; range 1.41–86.1 ng/mL) in 84% of the patients ($n = 38$). At 30 minutes after the procedure, the cTnI concentration had declined, with only 8.9% of the patients ($n = 4$) showing concentrations above the cutoff value (mean 9.45 ng/mL; range 2.83–18.5 ng/mL). The cTnI concentrations measured at these two times were significantly different ($p < 0.00001$). The cTnI concentration at 1 and 6 hours after angiography were both below the cutoff in all the 45 patients (Figure 1). The mean cTnI concentration at 5 minutes was higher in patients undergoing coronary intervention than in those undergoing diagnostic angiography only (27.2 vs 11.9 ng/mL; $p = 0.0385$). In the second *in vivo* study, we compared Opus Magnum and ACCESS estimated cTnI concentrations in samples taken from 25 patients. Unlike the results obtained by the Opus Magnum system, all ACCESS estimated cTnI concentrations were below the cutoff value of 0.15 ng/mL. There was no significant change in ACCESS estimated cTnI values of samples taken at 5 minutes and at 30 minutes, when comparing those values with baseline values ($p = 0.498$ and $p = 0.107$, respectively). The Opus Magnum estimated cTnI values, however, were found to have risen significantly at 5 minutes and 30 minutes, when compared with baseline values ($p = 0.001$ and $p = 0.002$, respectively). Of this patient group, 84% (21 patients) had elevated cTnI concentrations, as measured by the Opus Magnum system (Table 1). Opus Magnum estimated cTnI values at 5 minutes and 30 minutes were significantly different from those estimated by ACCESS at the same sample collection times ($p = 0.001$ and $p = 0.04$, respectively).

In the first *in vitro* study, we found that use of almost all of the contrast media resulted in a positive reaction for cTnI in the Opus Magnum assay. An average value was 13.1 ng/mL, and the maximum was found in the test using iodized ethylesters of poppy-seed oil, which had a value of 34.6 ng/mL. However, when using the ACCESS assay, iodized ethylesters of poppy-seed oil was the only contrast medium that produced a positive cTnI reaction (Table 2).

In the second *in vitro* study, in which we observed the effect of different concentrations of a contrast medium, both assays found the cTnI levels in pure serum to be below cutoff values. The cTnI concentration in the positive control serum, which came from a patient with AMI, was found by Opus Magnum to be 24.05 ng/mL and by ACCESS assay to be 23.93 ng/mL. When using the Opus Magnum assay to measure cTnI concentrations in the samples with different contrast medium-to-blood ratios (2–8%), we found that all concentrations were higher than the cutoff. The higher the ratio of contrast mixture to blood, the higher the estimated cTnI concentration. However, when the ACCESS assay was used to check the same specimens, the cTnI concentrations were found to be below the cutoff value (Table 3).

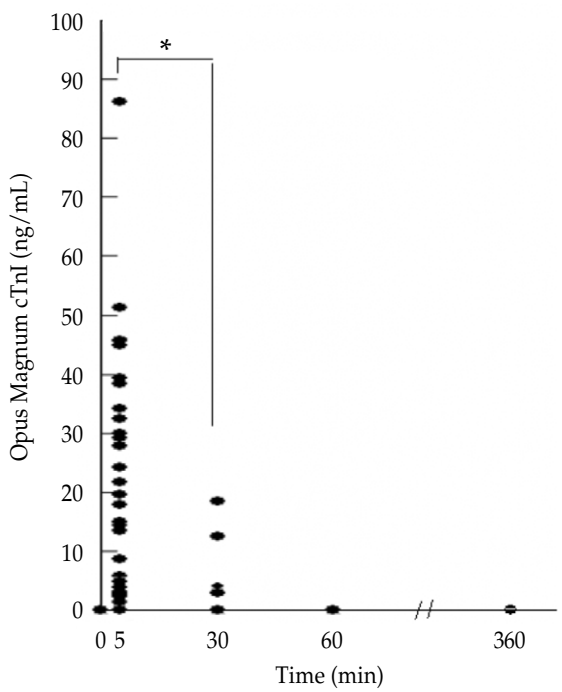

Figure 1. *The decay in the concentrations of cardiac troponin I (cTnI) by Opus Magnum assay in the* in vivo *study. Opus Magnum cTnI values declined significantly at 30 minutes compared with values at 5 minutes after coronary angiography. *p < 0.00001.*

Table 1. Results of 25 patients' cTnI by Opus Magnum and ACCESS systems

	Opus Magnum system, ng/mL			ACCESS system, ng/mL		
	Before CAG	5 minutes after CAG	30 minutes after CAG	Before CAG	5 minutes after CAG	30 minutes after CAG
1	< 0.5	42.0	22.0	0.013	0.016	0.015
2	< 0.5	8.9	< 0.5	0.022	0.007	0.021
3	< 0.5	50.0	< 0.5	0.016	0.038	0.037
4	< 0.5	17.58	4.8	0.001	0.069	0.066
5	< 0.5	2.5	< 0.5	0.016	0.038	0.027
6	< 0.5	3.53	< 0.5	0.044	0.050	0.061
7	< 0.5	3.5	< 0.5	0.018	0.029	0.062
8	< 0.5	19.6	< 0.5	0.021	0.016	0.016
9	< 0.5	< 0.5	< 0.5	0.033	0.032	0.026
10	< 0.5	8.02	< 0.5	0.028	0.037	0.074
11	< 0.5	< 0.5	< 0.5	0.029	0.036	0.029
12	< 0.5	< 0.5	< 0.5	0.011	0.011	0.015
13	< 0.5	15.0	< 0.5	0.034	0.025	0.035
14	< 0.5	49.5	37.3	0.022	0.050	0.014
15	< 0.5	45.0	25.0	0.021	0.015	0.025
16	< 0.5	3.48	< 0.5	0.016	0.011	0.014
17	< 0.5	0.55	< 0.5	0.001	0.001	0.082
18	< 0.5	48.6	< 0.5	0.021	0.012	0.038
19	< 0.5	1.16	0.784	0.024	0.021	0.022
20	< 0.5	1.59	1.46	0.069	0.046	0.024
21	< 0.5	1.03	0.711	0.014	0.006	0.016
22	< 0.5	1.02	< 0.5	0.025	0.019	0.021
23	< 0.5	< 0.5	< 0.5	0.026	0.011	0.033
24	< 0.5	0.639	0.688	0.031	0.009	0.039
25	< 0.5	8.7	0.858	0.111	0.127	0.111

CAG = coronary angiography.

Table 2. The cTnI assay results of 12 contrast media and one positive control serum

Contrast media	cTnI by Opus Magnum, ng/mL	cTnI by ACCESS, ng/mL
Conray60	11.30	0
Optiray320	11.10	0
Omnipaque300	12.90	0
Omnipaque350	14.60	0
Hypaque76	10.00	0
Angiografin60	7.47	0
Magnevist	10.90	0
Urografin76	8.44	0
Ultravist300	13.20	0
Ultravist370	12.90	0
Biligrafin	9.55	0
Lipiodol	34.60	0.54
Positive control	24.08	24.00

cTnI = cardiac troponin I.

Discussion

This study found that most contrast media can potentially lead to false-positive cTnI results in the Opus Magnum assays. Iodized ethylesters of poppy-seed oil can cause false-positive results in both assays. False-positive results can be observed up to 1 hour after coronary angiography.

Immunoassays use an array of mammalian antibodies, either monoclonal (mouse) or polyclonal (goat, sheep, rabbit). The Opus Magnum assay uses a fluorescence enzyme immunoassay as the test module. In one study, two different goat antibodies were used in the Opus Magnum's sandwich format [7]. One polyclonal antibody was in solid phase and bound to cTnI in the sample, and the other conjugated polyclonal antibody, which was coupled to fluorescence as a signal transducer, was detected after the antigen binding and the separation of the bound

Table 3. cTnI concentration in six samples with the Ultravist370 blood mixtures

Tube number	Ultravist370/blood volume ratio, %	cTnI by Opus Magnum, ng/mL	cTnI by ACCESS, ng/mL
1	0	< 0.5	0
2	2.0	4.32	0
3	4.0	4.44	0
4	6.0	4.53	0
5	8.0	7.72	0
6	Positive control	24.05	23.93

cTnI = cardiac troponin I.

form from the unbound form. The lack of binding specificity of reagent antibodies in either of the two reactions was found to cause interference [8]. The ACCESS cTnI assay is also a two-site immunoenzymatic (sandwich) immunoassay. All reagent antibodies in ACCESS are mouse monoclonal antibodies [9]. There may be an unidentified antigenic site on the contrast medium molecule for the reagents of polyclonal or monoclonal antibodies in the cTnI immunoassays. The difference in specificity between polyclonal and monoclonal antibodies may partly explain the higher prevalence of interference in the Opus Magnum assay than in the ACCESS assay.

cTnI is a new generation of cardiac markers and is generally accepted as a reliable tool for the diagnosis of AMI and for determination of risk for patients with acute coronary syndrome. However, cTnI may be increased in conditions other than cardiac injury. High cTnI values have been observed among patients with chronic renal failure or skeletal muscle damage [8]. In addition, several factors have been found to interfere with cTnI assay results. These include hemolysis, bilirubin, lipid, autoantibodies, heterophilic antibodies, rheumatoid factors, human anti-mouse antibodies, and heparin [4–6,10]. Negative interference factors have been less frequently reported. Hemodilution by fluid therapy [11], the presence of fibrin after incomplete separation of serum, circulating cTnI autoantibodies, bilirubin, hemoglobin, as well as some unidentified components in the blood of either healthy subjects or patients with acute coronary syndrome have all been reported to cause false-negative or falsely low results in certain cTnI assays [12]. We know of no study that has reported interference of contrast media with cTnI immunoassay results. Our *in vivo* study found false-positive cTnI results in 84% of our patients receiving coronary angiography when the Opus Magnum system was used.

Because of the rapid development of interventional cardiology, early coronary intervention is gradually becoming more common in the treatment of patients with acute coronary syndrome. Furthermore, in hospitals that are sufficiently equipped, primary coronary intervention is becoming a standard method of gaining reperfusion for patients with AMI. cTnI is used as a marker of cardiac injury after percutaneous coronary intervention. In this kind of intervention, the use of contrast media is essential. In our *in vivo* study, the potential interference of contrast media on assays for cTnI was stronger in patients receiving coronary intervention than in patients receiving coronary angiography alone. This result might be the consequence of the larger amounts of contrast media used. In any case, it is important for clinicians to recognize that many factors, including contrast media, can cause false-positive cTnI immunoassay results. The positive interference caused by contrast media in patients with normal renal function lasted for only 30 minutes in this study. In patients with some renal function impairment, it is possible that the positive interference can last longer than 30 minutes [13]. If cTnI levels are checked after a percutaneous coronary intervention or coronary angiography, the timing of the blood sampling is important. The interference by the contrast media can effectively be avoided if samples are collected 1 hour or longer after coronary angiography in patients with normal renal function. Such interference can be avoided in this way, even with a cTnI immunoassay such as Opus Magnum.

We used heparin at the beginning of coronary angiography, and a supplemental dose was usually needed during coronary intervention. The effect of heparin on cTnI has been investigated in several studies. The concentrations of troponin measured in heparin-treated plasma are markedly lower than in serum [14]. Therefore, the influence of heparin on the positive interference of contrast media on assay for troponin warrants further investigation.

There are several limitations in this study. First, the amount of contrast media used for each patient was incompletely recorded, so the actual correlation of interference and the volume of contrast media cannot be determined in this *in vivo* study. Second, the blood concentration of contrast media at 30 and 60 minutes after administration was not checked, so its effect on the magnitude of interference is uncertain.

Conclusions

The positive interference from various contrast media in cTnI immunoassays should be taken into consideration when interpreting cTnI values, especially when the blood samples are obtained within the first hour of angiography.

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顯影劑對心肌旋轉蛋白 I 免疫分析測定的偽陽性干擾

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心肌旋轉蛋白 I 是敏感性與可信度高的心肌受損指標，此外，心肌旋轉蛋白 I 的上升也是急性冠心症病人高風險的指標。由於應用免疫法，心肌旋轉蛋白 I 的濃度測定容易受到干擾。急性冠心症的冠狀動脈介入性治療必須使用顯影劑，然而，顯影劑是否會造成心肌旋轉蛋白 I 測定上的干擾，仍是未知的。本實驗的目的在要探討顯影劑對心肌旋轉蛋白測定的干擾。第一階段我們收集了四十五位接受冠狀動脈攝影的病人的血液檢體，採血的五個時間點分別為心導管術前、術後 5 分鐘、30 分鐘、60 分鐘和術後 6 小時。各檢體分別用 Opus Magnum 系統來測定心肌旋轉蛋白 I 的濃度。第二階段我們又另外收集 25 個病人的檢體同時用 Opus Magnum 與 ACCESS 系統做測定。體外實驗方面，我們用 12 種不同的顯影劑當成檢體，用 Opus Magnum 和 ACCESS 兩種系統來測量心肌旋轉蛋白 I 的值，以觀察是否顯影劑會直接造成系統的干擾。我們也製造不同比例的顯影劑混合血液，來評估干擾的強度與顯影劑的關係。我們發現心肌旋轉蛋白 I 的濃度在心導管術後 5 分鐘遠高於 30 分鐘，而所有上升的現象在術後 60 分鐘都消失。在第二階段的體內實驗發現兩種檢測系統之間有顯著的不同，所有 ACCESS 系統測出的 cTnI 值都低於正常值。在體外實驗中，所有 12 種顯影劑都會對 Opus Magnum 系統產生偽陽性的干擾，而只有 Lipiodol 這種顯影劑會造成 ACCESS 系統的干擾。顯影劑在血液中的比例愈高，以 Opus Magnum 系統測到的心肌旋轉蛋白 I 的值也愈高，而ACCESS 系統並沒有這種現象。顯影劑會造成心肌旋轉蛋白 I 測定法的偽陽性干擾。我們在判讀心導管使用顯影劑的心導管術後的病人的心肌旋轉蛋白 I 時，應該注意可能的干擾現象。

關鍵詞：免疫測定法，干擾，心肌旋轉蛋白 I，顯影劑

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