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*Original Article*

# Increase in cardiac myosin binding protein-C plasma levels is a sensitive and cardiac-specific biomarker of myocardial infarction

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**Abstract:** Earlier studies have shown that cardiac myosin binding protein-C (cMyBP-C) is easily releasable into the circulation following myocardial infarction (MI) in animal models and patients. However, since its release kinetics has not been clearly demonstrated, no parameters are available to judge its efficacy as a *bona fide* biomarker of MI in patients with MI. To make this assessment, plasma levels of cMyBP-C and six known biomarkers of MI were determined by sandwich enzyme-linked immunosorbent assay in patients with MI who had before and after Percutaneous Transcoronary Angioplasty (PTCA), as well as healthy controls. Compared to healthy controls (22.3 ± 2.4 ng/mL (n=54)), plasma levels of cMyBP-C were significantly increased in patients with MI (105.1 ± 8.8 ng/mL (n=65), $P<0.001$). Out of 65 patients, 24 had very high levels of plasma cMyBP-C (116.5 ± 13.3 ng/mL), indicating high probability of MI. Importantly, cMyBP-C levels were significantly decreased in patients (n=40) at 12 hours post-PTCA (41.2 ± 9.3 ng/mL, $P<0.001$), compared to the patients with MI. Receiver operating characteristic analysis revealed that a plasma cMyBP-C reading of 68.1 ng/mL provided a sensitivity of 66.2% and a specificity of 100%. Also, myoglobin, carbonic anhydrase and creatine kinase-MB levels were significantly increased in MI patients who also had higher cMyBP-C levels. In contrast, levels of cardiac troponin I, glycogen phosphorylase and heart-type fatty acid binding protein were not significantly changed in the samples, indicating the importance of evaluating the differences in release kinetics of these biomarkers in the context of accurate diagnosis. Our findings suggest that circulating cMyBP-C is a sensitive and cardiac-specific biomarker with potential utility for the accurate diagnosis of MI.

**Keywords:** Acute coronary syndrome, cardiac biomarker, cardiac myosin binding protein-C, contractile protein, cMyBP-C, myocardial infarction

## Introduction

Acute coronary syndrome (ACS) is a major cause of morbidity and mortality worldwide, and it incurs huge healthcare expenditures [1-4]. In the United States alone, 683,000 discharge occurrences resulting from ACS were reported in 2009 [5, 6]. Remarkably, 1,190,000 secondary discharges were associated with ACS, of which 829,000 were attributed to myocardial infarction (MI) alone [5, 6]. ACS is caused by the sudden obstruction of a coronary artery, and the primary symptom is acute chest pain with additional symptoms, such as sweating, nausea, vomiting, and shortness of breath. Early diagnosis and prompt treatment of ACS continues to be a diagnostic challenge in medicine, especially since ACS constitutes a large spectrum of clinical conditions, such as unstable angina (UA, absence of MI), non-ST elevation MI (NSTEMI), and acute ST-elevation MI (STEMI). These three categories are normally classified based on the presence or absence of ST segment elevation on the electrocardiogram and increased plasma levels of myocardial biomarkers. However, blood levels of the current gold standard cardiac biomarkers are very low during the initial stages of MI, making early diagnosis difficult. This results in patients waiting for 6 to 12 hours for accurate clinical differ-

entiation between UA and NSTEMI before the appropriate treatment method can be chosen. Therefore, a robust biomarker able to determine the presence and the severity of MI at the earliest onset (0-6 hours) is urgently needed.

The timing and concentration of currently preferred diagnostic biomarkers, such as circulating cardiac troponin I and T (cTnI, cTnT), depend on local blood flow, infarct-related artery patency, infarct size, individual biomarker localization, molecular weight, and half-life (clearance rate) in the blood. Nonspecific cTnI false-positive results are often reported among hospitalized patients and can easily lead to incorrect prognoses [7]. Recent American College of Cardiology/American Heart Association guidelines report high mortality among patients with MI in the absence of cTnI, indicating that measurement of plasma cTnI levels is not necessarily an accurate evaluation of atherosclerotic coronary artery disease [8-10]. Studies have shown that assessment of heart-type-fatty acid-binding protein (H-FABP) within the first 4 hrs of MI is superior to cTnT [11] and that testing for both H-FABP and cTnI is a reliable tool for the early diagnosis of MI/ACS, as well as a valuable rule-out test for patients presenting at 3 to 6 hours after chest pain onset [12]. Cardiac myosin binding protein-C (cMyBP-C) is a cardiomyocytespecific sarcomeric assembly protein, which regulates both sarcomeric structure and function.

We previously reported that cMyBP-C is a potential biomarker of MI by its dephosphorylation, proteolysis and subsequent release into the circulation post-MI in a rat model and patients with MI [13]. However, since the number of subjects in this human study was limited to fifteen, the results were insufficient to confirm whether cMyBP-C is a *bona fide* biomarker of MI equal to, or exceeding, the diagnostic performance currently preferred biomarkers, including the gold standard cTnI and cTnT, as noted above.

Therefore, the aim of the present study was to determine the level of cMyBP-C compared to the currently preferred diagnostic biomarkers and define the level of cMyBP-C in patients with MI after Percutaneous Transcoronary Angioplasty (PTCA), compared to the baseline levels in healthy controls. Our findings suggest that circulating cMyBP-C is a sensitive and cardiac-specific biomarker with potential utility for the accurate diagnosis of MI.

## Methods

### *Human samples*

Categorization of ACS followed the guidelines of the American Heart Association/American College of Cardiology [8-10]. A total of 65 patients (aged 62 ± 15 years; 52 males and 13 females) were admitted to the ER (Base, MI) with complaint of acute coronary syndrome. Of these patients, a total of 40 underwent Percutaneous Transcoronary Angioplasty. Samples from all 65 patients were measured to determine the level of plasma cMyBP-C, and those subjects undergoing PTCA were measured 12 hours post-procedure. A diagnosis of MI was determined by the presence of STEMI. These samples were previously procured in a subclinical study to determine the inflammatory response to Brachytherapy following PTCA. For negative controls, 54 plasma samples were obtained from volunteers (aged 25 ± 20 years; 32 males and 22 females). The Institutional Review Board at Loyola University Chicago approved the protocol for the use of de-identified human samples previously used for research studies. No other sample information was available to the investigators or technicians in the laboratory.

### *Sandwich ELISA assay for cMyBP-C*

Plasma level of cMyBP-C was determined by sandwich ELISA as described previously [13]. Reproducibility, variability, and detection limits were tested by coating Sigma-Nunc-Immuno™ MicroWell™ 96 MaxiSorp™ polystyrene solid plates (Catalog # 449824, Thermo Scientific Nunc, USA) overnight with capture mouse monoclonal antibody raised against cMyBP-C C0 region (Catalog # sc-137180 Clone E7, Santa Cruz Biotechnology, Santa Cruz, CA). The unbound capture antibody was removed by washing with phosphate buffered saline (PBS)-Tween 20 (0.01%) using an ELISA plate washer (Immuno wash® 1575, Bio-Rad, Hercules, CA). The plate was then blocked using 1x-blocking buffer (Catalog # 11921673001, Roche Diagnostics Corp., Indianapolis, IN 46250) and incubated on an ELISA plate shaker at 200 rpm/min (IKA-Werke, Germany) for 1 hour at room temperature. Subsequently, the plates

were washed, as detailed earlier, and a dilution series (recombinant mouse cMyBP-C N'-terminal peptide 40 kDa), ranging from 0.096 ng/ml to 1500 ng/ml, was used to obtain standard curves. The plate was washed as detailed earlier. As detection antibody, rabbit polyclonal cMyBP-C (residues 2-14) at 1:1000 dilution in PBS was used [13]. The plate was then incubated for 1 hour at room temperature, while shaking at 200 rpm/min. The plate was washed again, and HRP-conjugated donkey anti-rabbit IgG antibody (Catalog # sc-2313, Santa Cruz Biotechnology, Santa Cruz, CA) was added at a dilution of 1:2000 in PBS, followed by 30 min incubation, while shaking at 200 rpm/min at room temperature. The plate was washed again, and for signal detection, 1-step 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, Catalog # 37615, Thermo Scientific, USA) was added. The plate was incubated at 37°C for 5 minutes. The absorbance was measured at 405 nm using the BioTek Epoch microplate reader. The data were analyzed using the Gen5™ 5.1.11 Data Analysis Software (BioTek). Standard curve was fitted with a 4-parameter curve at $1/y^2$ weighting.

*Multiplex assay for existing cardiac biomarkers*

A six-plex determination of existing cardiac biomarkers, cTnI, MYO, CK-MB, GPBB, CAIII, and H-FABP, was performed using the Randox biochip array technology [14] on the Evidence Investigator analyser (Catalog # EV3602, Randox Laboratories, Crumlin, United Kingdom). The Cardiac Plus Array kit and Cardiac Plus control were used according to the manufacturer's instructions (Catalog # EV3511, EV3558, Randox Laboratories Limited, Crumlin, United Kingdom).

*Statistical analysis*

The data are represented as percent of mean ± standard error of the mean (SEM). Comparisons between groups (Base MI, 12 hrs post-PTCA and control) were made using pairwise multiple comparison procedures (Holm-Sidak method, Sigma Plot, Systat Software, San Jose, CA). A value of $P<0.001$ was considered statistically significant. One-way ANOVA analysis was used to compare the level of cMyBP-C with other biomarkers. Receiver operating characteristic (ROC) curve was generated by plotting sensitivity versus specificity to assess the possible predictive value of each cMyBP-C value in classifying patients into positive or negative status (GraphPad Prism 5, La Jolla, CA).

## Results

*Plasma cMyBP-C level is elevated in patients with MI*

cMyBP-C is a predominant cardiomyocyte-specific structural protein that regulates cardiac structure and function [15-18]. Importantly, cMyBP-C differs from its skeletal isoforms by having an N'-terminal C0 domain (99 residues) and being exclusively expressed in the heart (**Figure 1**) [19]. Using the first 14 residues of cMyBP-C-specific C0 domain, we previously generated a rabbit polyclonal (cMyBP-C$^{2-14}$) antibody for detection in the sandwich ELISA assay [13]. The capture mouse monoclonal antibody was generated against the entire C0 domain (Clone E7, Santa Cruz, CA). Sandwich ELISA was previously developed using both of these antibodies to determine the level of cMyBP-C in either serum or plasma samples [13]. The sandwich ELISA was characterized by comparing actual (spiked) and expected (calculated) values to determine coefficient of variation, recovery percentages, dynamic range and reproducibility of the assay (**Table 1**).

Sandwich ELISA data showed that plasma level of cMyBP-C is significantly elevated in MI samples (105.08 ± 8.80 ng/mL, $P<0.0001$, n=65), compared to control samples (22.27 ± 2.36 ng/mL, n=54, **Figure 2A-C**). A 4.78-fold increase in plasma cMyBP-C levels was observed in patients with MI, compared to the controls. Samples of these patients were collected at the time of their admission to the ER. Furthermore, at 12 hours post-PTCA, the level of cMyBP-C was significantly decreased in patients who underwent the minimally invasive procedure (41.21 ± 9.30 ng/mL). Although the level was still significantly higher than the controls, the fold change was decreased from 4.78 to 1.88, relative to the base MI. Interestingly, 24 MI patients out of 65 MI patients presented with higher plasma cMyBP-C levels (116.53 ± 13.32 ng/mL) with a minimal value of 45.45 ng/mL. In total, sixty-six percent of MI samples had higher cMyBP-C levels than the control group, a datum that is also reflected in the ROC curve (**Figure 2D**). Importantly, the ROC curve showed that a cMyBP-C plasma level of 68.1

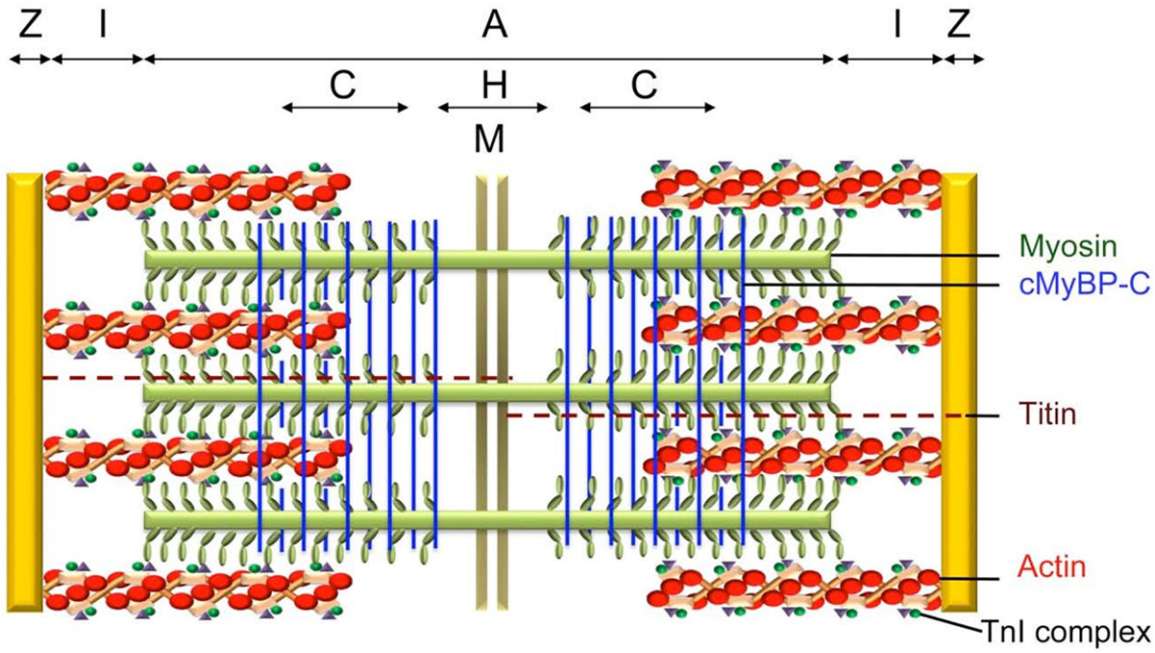


**Figure 1.** Schematic structure of sarcomere showing the localization of cardiac myosin binding protein-C (cMyBP-C). The basic functional unit of cardiac muscle is the sarcomere, which consists of both thin and thick filament proteins. Thick filament proteins are titin, myosin and cMyBP-C, whereas thin filament proteins are actin, α-tropomyosin and troponin complex that includes troponin C, I and T. The increased level of cTnI and cTnT in the blood has been extensively characterized, and both indicators are the current gold standard biomarkers of MI. However, the release of other sarcomeric proteins has not been completely characterized. Particularly, cMyBP-C forms 7-9 transverse stripes at regular intervals of 43 nm in the C-zone of the sarcomeric A-band, which contribute to 2% of total myofibril mass. Unlike cTnI and cTnT, cMyBP-C protein belongs to the intracellular immunoglobulin (Ig) superfamily.

**Table 1.** Analytical parameters of sandwich ELISA in quantitating plasma cMyBP-C levels

| Theoretical value of calibrators (ng/ml) | Calculated mean values (ng/ml) | Standard Deviation | Coefficient of Variation (%) | Recovery % |
|---|---|---|---|---|
| 0.096 | 0 | 0 | 0 | 0 |
| 0.48 | 0.58 | 0.167 | 28.6 | 121 |
| 2.4 | 2.15 | 0.36 | 16.8 | 90 |
| 12 | 12.76 | 0.75 | 5.9 | 106 |
| 60 | 57.94 | 2.93 | 5.1 | 97 |
| 300 | 308.7 | 44.4 | 14.4 | 103 |
| 1500 | 1564.2 | 131.7 | 8.4 | 105 |

The detection values are as follows: LLOD (1.41 ± 0.39 ng/ml), LLOQ (2.4 ng/ml), ULOD (1500 ng/ml) and ULOQ (1500 ng/ml). The intra- and interplate variability are 6.2 ± 2.1% and 13.3 ± 4.4%, respectively (triplicates).

ng/mL is predictive of MI with specificity of 100% and sensitivity of 66.2% (area under curve (AUC) of 0.89).

*Comparison between cMyBP-C levels and those of other cardiac biomarkers*

Next, twenty-four samples that represented base MI with high levels of cMyBP-C (116.53 ± 13.32 ng/mL) were further used for simultaneous determination of plasma levels of cTnI, MYO, CK-MB, GPBB, CAIII, and H-FABP with a biochip-multiplexing assay (**Table 2**). Quantification of plasma levels of all the cardiac biomarkers in this study is summarized in **Table 3**. Levels of cMyBP-C, CK-MB, MYO, and GP-BB were all significantly higher in MI patients compared to control samples ($P<0.001$). Elevated levels of plasma cTnI, H-FABP, CKMB and GP-BB were observed, but they were not significantly higher than control. Strikingly higher than the controls in these 24 samples, the mean value

**Table 2.** Functional sensitivity and limit of detection of other biomarkers with the cardiac biochip array (multiplex) as provided by the manufacturer. The respective cut off values and release kinetics are also indicated

| Calibrators | LLOD (ng/ml) | ULOD (ng/ml) | Cut off value Normal (ng/l) | Cut off value MI (ng/ml) | Onset (hrs) | Peak (hrs) | Duration (hrs) | Reference |
|---|---|---|---|---|---|---|---|---|
| GP-BB | 1.97 | 200 | | | 2-4 | 8-12 | 24-36 | [40, 41] |
| CAIII | 0.2 | 200 | Skeletal Muscle Only | | | | | |
| MYO | 1.8 | 700 | 6-85 | 450 | 1-4 | 6-7 | 18-24 | [42, 43] |
| CK-MB | 0.4 | 100 | 3-4 | 12 | 3-12 | 14-16 | 24-36 | [43-45] |
| H-FABP | 0.15 | 100 | 0 | 5 | 1-3 | 6 | 18-30 | [43, 45, 46] |
| cTnI | 0.18 | 50 | 0 | 5 | 3-12 | 18-24 | 216 | [43, 47] |

Carbonic anhydrase III (CAIII) is expressed exclusively in skeletal muscle and restricted to skeletal muscle injury in association with MI [48].

**Table 3.** Comparison of plasma cMyBP-C levels with other biomarkers of MI. The values are expressed as mean values ± SEM values (ng/ml). Data show that plasma levels of cMyBP-C in patients with MI at base are significantly higher, compared to controls, for the selected 24 samples presenting high levels of cMyBP-C (>45.45 ng/mL)

| | cMyBP-C (ng/ml) | | cTnI (ng/ml) | | CK-MB (ng/ml) | | MYO (ng/ml) | | GP-BB (ng/ml) | | H-FABP (ng/ml) | | CAIII (ng/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Base (MI) | Control | Base (MI) | Control | Base (MI) | Control | Base (MI) | Control | Base (MI) | Control | Base (MI) | Control | Base (MI) |
| Mean (ng/ml) | 18.51 ± 2.10 | 116.53 ± 13.32 | 0.19 ± 0.02 | 0.22 ± 0.01 | 0.66 ± 0.08 | 2.10 ± 0.4 | 6.09 ± 1.64 | 40.78 ± 5.13 | 6.11 ± 1.38 | 10.17 ± 1.46 | 1.97 ± 0.9 | 3.13 ± 0.46 | 4.40 ± 1.43 | 26.28 ± 4.61 |
| Fold change | | 6.3 | | 1.15 | | 3.18 | | 6.69 | | 1.66 | | 1.58 | | 5.97 |
| *P*-value | | <0.001 | | 0.14 | | 0.00 | | <0.001 | | 0.05 | | 0.25 | | <0.001 |
| Min (ng/ml) | 1.81 | 45.45 | 0.11 | 0.18 | 0.4 | 0.4 | 0.4 | 14.81 | 2.00 | 2.00 | 0.15 | 1.12 | 0.20 | 9.35 |
| Max (ng/ml) | 38.04 | 236.36 | 0.36 | 0.31 | 1.61 | 9.90 | 39.8 | 60.96 | 20.91 | 20.54 | 17.62 | 11.91 | 34.89 | 86.07 |
| n | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |

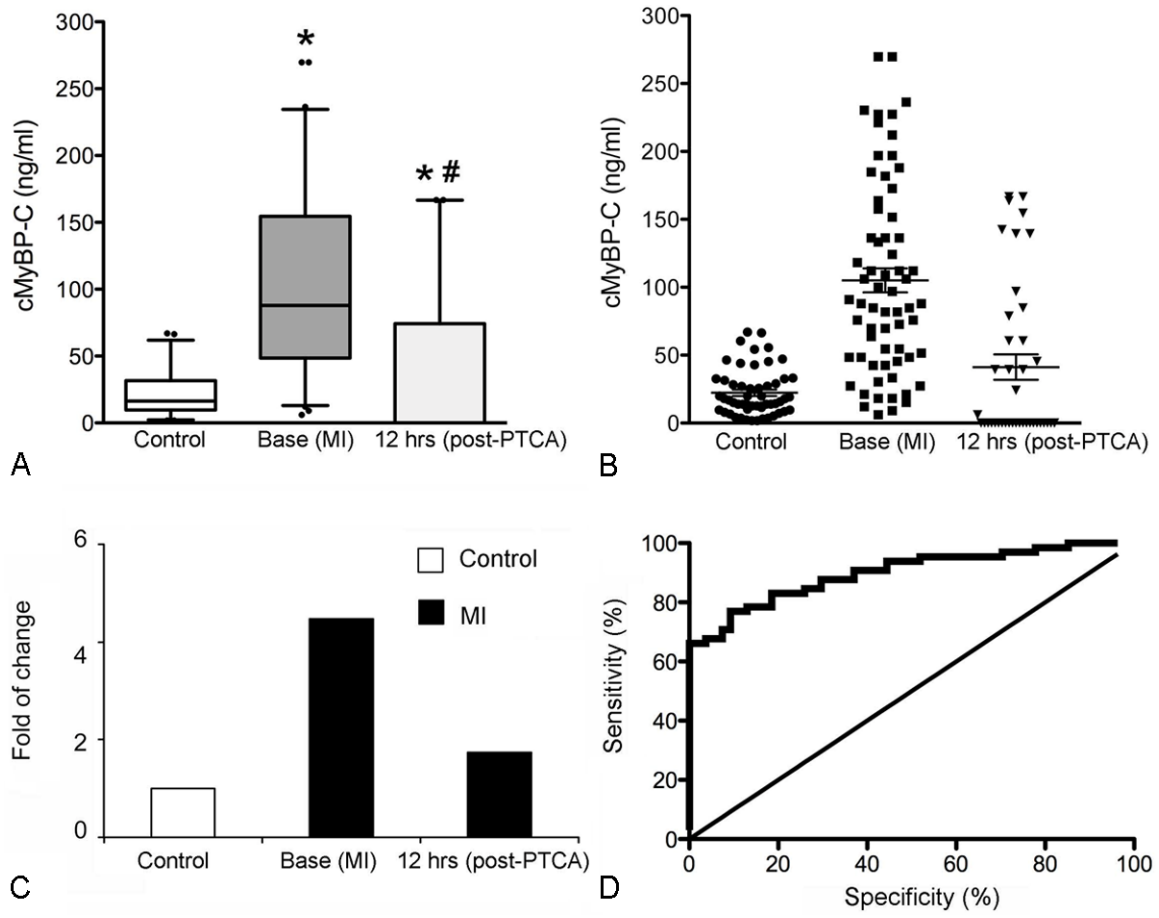


**Figure 2.** Elevated level of plasma cMyBP-C in patients with MI. Data are shown as box-andwhisker plots of cMyBP-C measured in MI patients prior to PTCA (Base, n=65) and 12 hrs post-PTCA (n=40), in comparison with healthy control samples (n=54, A). *$P$<0.001 versus control; #$P$<0.001 versus base. Distribution of plasma cMyBP-C levels in control samples and MI samples (B). cMyBP-C level was measured in control, Base (MI) and 12 hrs post-PTCA (MI) groups as shown in panel A, and data are displayed for individual patients. Line and whiskers: 22.27 ± 2.36 ng/ml and 105.08 ± 8.80 ng/ml. Fold change of cMyBP-C level was determined in Base (MI) and 12 hrs post-PTCA (MI) groups, compared to controls (C). ROC curve for plasma cMyBP-C levels comparing MI patients (n=45) and controls (n=45, D). Area under the curve (AUC)=0.89, 95% CI: 0.83 to 0.95. cMyBP-C plasma level of 68.1 ng/ml has 100% specificity and 66.2% sensitivity.

of cMyBP-C was predominant and robust at 116.53 ± 13.32 ng/mL, as shown in **Figure 3** and **Table 3**. None of these six biomarkers had higher protein concentration in the circulation than cMyBP-C, indicating that increased plasma level of cMyBP-C is a strong determinant for the presence of MI.

## Discussion

cMyBP-C is a new and promising cardiac-specific marker for the early detection of MI [20-23]. It is a thick filament assembly protein in the sarcomere, which interacts with titin, myosin and actin to regulate the structure and function of the heart. Phosphorylation of cMyBP-C at Ser-273, Ser-282, and Ser-302 regulates myocardial function and confers resistance to proteolysis, preserving cardiac function post-MI [24-28]. In contrast, dephosphorylation at Ser-273 and Ser282 facilitates cMyBP-C degradation and the release of a 40 kDa N'-terminal fragment, which is toxic to cardiomyocytes and significantly impairs contractility and $Ca^{2+}$ handling via inhibition of actomyosin function [19]. In fact, our previous studies have demonstrated that cMyBP-C is easily soluble and releasable from the sarcomere [21]. Moreover, the large size of cMyBP-C and its early release into the circulation in response to ischemic injury make it an ideal biomarker candidate for early detection of MI [29]. The present study, more-

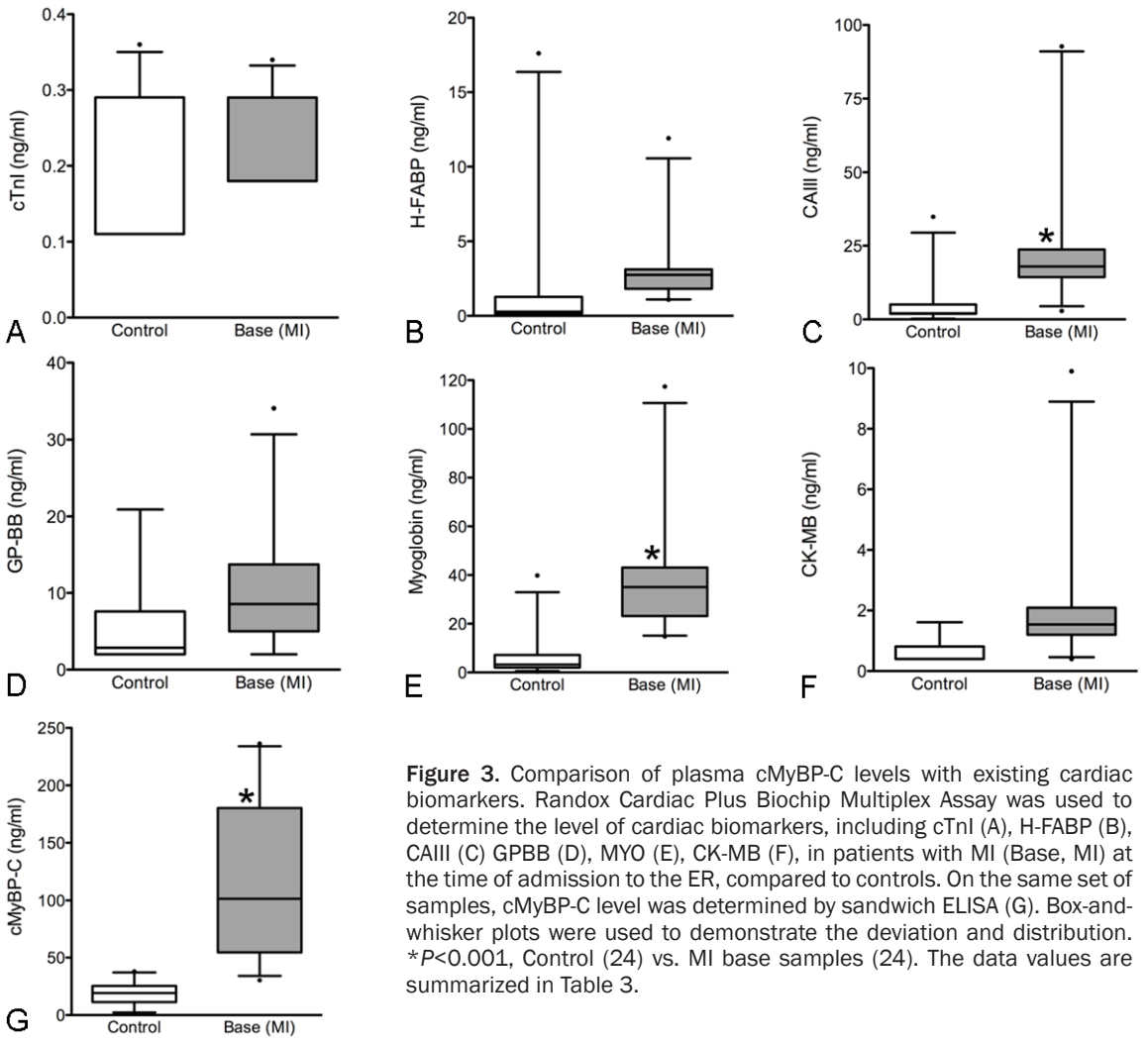


**Figure 3.** Comparison of plasma cMyBP-C levels with existing cardiac biomarkers. Randox Cardiac Plus Biochip Multiplex Assay was used to determine the level of cardiac biomarkers, including cTnI (A), H-FABP (B), CAIII (C) GPBB (D), MYO (E), CK-MB (F), in patients with MI (Base, MI) at the time of admission to the ER, compared to controls. On the same set of samples, cMyBP-C level was determined by sandwich ELISA (G). Box-and-whisker plots were used to demonstrate the deviation and distribution. *$P$<0.001, Control (24) vs. MI base samples (24). The data values are summarized in Table 3.

over, confirmed that cMyBP-C could act as a robust biomarker for MI in patients with ACS. In addition, the presence of N'terminal fragments of cMyBP-C in myocardial injuries can contribute to altered protein-protein interaction, impaired $Ca^{2+}$ handling, and contractile dysfunction [19]. Regulation at these phosphorylation sites may play an important role in the degradation and release kinetics of cMyBP-C, but further research is necessary to determine whether this fully explains the mechanism underlying cMyBP-C release into the circulation post-MI. We recently determined that after ligation of the coronary artery in adult swine, the levels of cMyBP-C were elevated in the circulation within 30 minutes post MI [30]. Importantly, we also determined that plasma cMyBP-C levels were significantly raised within 30 minutes in humans with hypertrophic cardiomyopathy undergoing transcoronary ablation of septal hypertrophy [30], suggesting that cMyBP-C is an *ultraearly* biomarker of MI.

When the plasma levels of cMyBP-C from patients were compared with the levels of other existing cardiac biomarkers of MI, such as cTnI, MYO, CK-MB, GP-BB, H-FABP, and CAIII, the increase of cMyBP-C was comparable or higher. Among the 65 MI patients studied, plasma levels of cMyBP-C declined 12 hours post-PTCA, indicating that cMyBP-C may be more useful than the gold standard biomarker cTnI, which is normally detected 6-12 hours after onset of MI and persists in the blood for more than 2 weeks. Diagnostic testing for MI under ER conditions (within 3-6 hours of onset) may benefit from combining several biomarkers to realize a synergistically more sensitive and specific "multimarker" regimen for the early and accurate detection of acute MI [31-34]. CK-MB and

the troponins do not show significantly increased sensitivity, but combinations of MYO with CK-MB [32, 33, 35] or troponins [32, 33] were promising. In a study of 6,352 chest pain patients, with 814 patients suffering from acute MI, MYO, CK-MB, or combination of these two were sensitive for acute MI only 64%, 52%, and 72%, respectively, with specificities of 90%, 96%, and 88% [36]. CK-MB, MYO, cTnI, and cTnT plasma markers all appear within 3-6 hours post-MI [37]. Studies have shown that assessment of heart-type-fatty acid-binding protein (H-FABP) within the first 4 hrs of MI is superior to cTnT [11], and testing with both H-FABP and cTnI is a reliable tool for the early diagnosis of MI/ACS and a valuable rule-out test for patients presenting at 3 to 6 hours after chest pain onset [12].

However, to increase the specificity of such "multimarker" regimens, it is advantageous to add a biomarker shown to be a comparatively robust, specific and sensitive determinant of MI. Furthermore, to reduce potential false-positive analytic results, the lowest cutoff of sensitivity and specificity of troponin assays should be set above the 99th percentile, with 10% coefficient of variation. This requirement can be attributed to the lack of precision in low concentration ranges of the biomarker [38]. Thus, very low cTnI levels in multiplexing may help exclude MI diagnoses, but cross validation with a more sensitive biomarker, such as cMyBP-C, will support a definitive diagnosis of MI based on the higher presence of detectable cMyBP-C in plasma. Both MI and control sample sizes were small in this study and a systematic time-point study is required to more precisely characterize the release kinetics of cMyBP-C post-MI. Nonetheless, the data derived from this study have sufficiently demonstrated that cMyBP-C is a sensitive and early cardiac-specific biomarker of MI, compared to the currently preferred diagnostic biomarkers, and may prove to be an extremely useful tool in providing rapid and accurate point-of-care diagnoses and treatments for ACS patients [29].

In summary, we have demonstrated that the robust release of cMyBP-C is useful as a sensitive, cardiac-specific biomarker of MI. In a study of MI patients with ACS, data confirmed that plasma cMyBP-C levels were significantly higher than those of cTnI, suggesting that cMyBP-C could be a more effective biomarker by its consistently increased titers over those of the gold standard marker cTnI. It was also suggested that the inclusion of cMyBP-C as a robust component of a "multimaker" test regimen could lead to more accurate and timely, i.e., within 3 to 6 hours, diagnosis of MI patients with ACS [30]. Protein release time-course experiments and exact cutoff values in healthy normal versus patients with MI will be required to ascertain whether cMyBP-C can provide greater sensitivity during earlier time points after MI, which is an ongoing project in our group [29, 30].

## Authors' note

A poster presentation of this study was given before the American College of Cardiology annual meeting, March 25, 2012, Chicago, IL, USA [39].

## Abbreviations

ABTS, 1-step 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ACS, acute coronary syndrome; AUC, area under the curve; α-TM, α-tropomyosin; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CAIII, carbonic anhydrase III; CK-MB, creatine kinase-MB; cMyBP-C, cardiac myosin binding protein-C; ELISA, enzyme-linked immunosorbent assay; GP-BB, glycogen phosphorylase BB; H-FABP, heart-type fatty acid-binding protein; IR, ischemia-reperfusion; LOD, limit of detection; LLOD, lower limit of detection; LLOQ, lower limit of quantitation; NSTEMI, non-ST elevation MI; ng/ml, nanogram/milliliter; MYO, myoglobin; MI, myocardial infarction; PBS, phosphate buffered saline; PTCA, Percutaneous Transcoronary Angioplasty; ROC, Receiver Operating Characteristic; SEM, standard error of mean; STEMI, ST-elevation MI; TASH, transcoronary ablation of septal hypertrophy; UA, unstable angina; ULOD, upper limit of detection; ULOQ, upper limit of quantitation.

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## Disclosures

A full patent application is pending (Application Serial No. 13/464,466, Pub. No. US 2012/0282618 A1 and Date: 05/04/12) to determine the risk factors associated with cMyBP-C degradation and release into human body fluid.

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