ORIGINAL ARTICLE

Diagnostic accuracy of cardiac myosin-binding protein C for acute myocardial infarction

Kambiz Masoumi, Arash Forouzan, Hassan Motamed, Habib Heybar, Nastaran Ranjbari and Sepideh Mohajer Shirazi

ABSTRACT

Background: Acute myocardial infarction (AMI) leads to increased mortality and recurrent ischemia and should be diagnosed promptly and distinguished from other causes of chest pain. It is necessary to evaluate the accuracy of cardiac myosin-binding protein (cMyC) to identify it a useful biomarker in the early diagnosis of AMI patients.

Methods: Participants diagnosed with AMI were confirmed based on clinical findings and electrocardiography and increased cardiac troponin 1 (cTn1) levels in the Emergency Department of Ahvaz Golestan Imam Khomeini Hospitals, Iran during the 2018 year. A complete blood count, serum glucose, cTn1, blood urea nitrogen, creatinine, sodium, potassium, and serum levels of cardiac myosin-binding protein (cMyC) were measured. The patients were followed up for 24 hours.

Results: Sixty-five patients with AMI were included in the study of whom 64.6% were ST-elevation myocardial infarction. The value of AUC = 1.00 (95% CI: 1.00-1.00) indicated that the CMyBP-C marker fully and correctly identified AMI individuals. At a significance level of 5%, the P-value <0.001 indicated that the CMyBP-C marker has an excellent ability to differentiate AMI.

Conclusion: Our study showed that the cMyC biomarker was significantly higher in AMI patients at all studied times and had a high diagnostic accuracy in diagnosing patients with AMI.

Keywords: cardiovascular disease; myocardial ischemia; acute myocardial infarction; myosin-binding protein c; diagnosis.

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INTRODUCTION

Acute myocardial infarction (AMI) leads to increased mortality and recurrent ischemia and should be diagnosed promptly and distinguished from other causes of chest pain (1). About 5 million emergency room visits occur each year in the USA due to chest pain. More than 800,000 patients experience acute myocardial infarction each year, and 27% of them die (2). However, since 1970, with advances in diagnostic testing and management of these patients, the mortality rate from acute myocardial infarction has decreased (3).

Diagnosis of patients with suspected AMI should focus on clinical history, physical evaluation, electrocardiography (ECG), cardiac markers, and chest X-ray (CXR) radiography (4). Characteristics of cardiac necrosis play a vital role in diagnosing AMI in individuals suspected of having non-ST-elevation acute coronary syndrome (NSTE-ACS) (5).

Cardiac troponins (cTn) have emerged as the gold standard for the diagnosis of AMI (6). Early cTn generations were not very sensitive to detecting low concentrations in the early hours following symptoms because cTn concentrations occur approximately 16 to 18 hours after the onset of symptoms of chest pain (7,8). Latest generation, high-sensitivity cTn measurement, attempt to generate low concentrations of cTn for the diagnosis and treatment of primary NSTE-ACS (9) and early reports suggested that this measurement would lead to a faster diagnosis of AMI (10). Therefore, due to limitations in sensitivity and specificity, guidelines recommend that diagnostic tests be repeated. Although many biomarkers are released rapidly during myocardial injury, none of them exceeds cTn, which is why these biomarkers are not explicitly expressed in the heart. As a result, researchers today focus on analyses that magnify periodic changes in serum cTn concentrations to improve its low positive predictive value (PPV) in the diagnosis of AMI (11).

However, it is unclear how this strategy can offer a straightforward utility (11). If so, what percentage of magnification and absolute change in concentration can overcome analytical noise and biological differences (12). In addition, magnification of changes in parameters, such as concentration in delta, gender, age, and vendor-specific, are likely to interfere with the alternatives (13). Therefore, an ideal marker should be analogous to the profile of systolic-released proteins, such as creatine kinase, fatty acid-binding protein, and myoglobin. Still, its expression should be specific to heart tissue.

Sarcomeric protein, cardiac myosin-binding protein (cMyC), is one of these candidates, which has been detected during systemic proteomic analysis of coronary secretions of rat heart during ischemia (14). c-Myc is a thick filament protein and one of the most abundant cardiac proteins. c-Myc has three different isoforms encoded by three different genes: slow skeletal, fast skeletal, and cardiac. The latter case has a unique N-terminal and specific characteristics and demonstrates cardiac epitopes (15). Recent studies have shown that this protein is elevated in patients with AMI. In addition to its high specificity for heart tissue, this marker is more abundant than cTn and is released into the bloodstream faster during AMI (16,17). Therefore, it is necessary to evaluate the accuracy of C-Myc diagnosis to identify it as useful biomarker in the early diagnosis of AMI patients.

METHODS

Study design

This study was conducted according to the Standards for the Reporting of Diagnostic accuracy studies (STARDs) guideline (18). A retrospective diagnostic accuracy was conducted after receiving ethics approval from the Ethics Committee of Ahvaz University of Medical Sciences (AJUMS; Number: IR.AJUMS.REC.1397.488). The study was conducted by the Declaration of Helsinki for research involving human subjects.

Participants

Participants diagnosed with AMI were confirmed based on clinical findings and ECG and increased cardiac troponin 1 (cTn1) levels. The patients gave written informed consent in the Emergency Department of the Ahvaz Golestan and Imam Khomeini Hospitals, Iran, during the 2018 year. Our control group consisted of patients with any problems other than signs and symptoms related to MI and final other diagnosis.

Inclusion criteria:

- Age over 18 years.
- Patients with ST-elevation MI (STEMI) and non-ST-elevation MI (NSTEMI).
- Complaints of chest pain, chest heaviness, and sweating, or any evidence of angina equivalent.

Exclusion criteria:

- Pregnant women.
- Acute heart failure or congestive heart failure.

Test methods

After monitoring the patient, a blood sample was taken and sent to the laboratory for the measurement of complete blood count, glucose, troponin, blood urea nitrogen, creatinine, sodium, potassium, and serum levels of cMyC. The troponin and cMyC levels were measured using TOYO (Turklab A.S, Izmir, Turkey) laboratory kitsets. Plasma level of cMyBP-C were measured by sandwich ELISA using the MYBPC3 kit.

Blood samples were taken at onset, 3 hours later, 6 hours later, 9 hours later) and sent to the laboratory to determine serum levels of troponin while cMyBP-C levels were followed-up for 24 hours. In the end, the patient's final condition (re-stroke) was recorded. Age, gender, and other demographic information were recorded.

Data analysis

The standard deviation of cMyBP-C at 6 hours was equal to 780 ng/L. Also, the error rate was 200 ng/L, and the sample size was calculated to be 60 people (19).

Quantitative variables were reported as mean, standard deviation, minimum, maximum, and qualitative variables registered as number (percentage). The normality of quantitative variables was assessed using the Shapiro-Wilk test. The Chi-square test was used to examine the relationship between qualitative variables, and the Pearson correlation coefficient or its nonparametric equivalent (Spearman) was used for quantitative variables. Comparing the time to reach the maximum amount of protein C bound to myosin of the heart and troponin in patients with AMI was performed using paired t-test or nonparametric equivalent (Wilcoxon test). The duplicate size test or the T-model, or the Generalized Estimating Equations (GEE) model were used to analyse the data in a multivariate manner. Sensitivity, specificity, and the area quantified diagnostic accuracy under the receiver operating curve (AUC [95% CI]) against adjudicated AMI.

RESULTS

A total of 65 patients with AMI were included in the study. The mean age of patients was 57.03 years ± 12.25 and 61.5% of patients were male. Regarding AMI type, 64.6% were STEMI. Other patient characteristics are presented in Table 1. CMyBPC biomarker changes at zero, 3, 6, 12, and 24 hours after the referral had increasing and decreasing fluctuations (Figure 1). If in all patients the mean score of CMyBPC had a significant decreasing trend from the time of referral to 9 hours and then at 12 hours the referral increased relatively compared to 9 hours and then decreased again at 24 hours (Figure 2). Changes in the Troponin I biomarker between zero and 24 hours after referral also varied according to the onset group. If the level of Troponin I in the group of patients with pain onset time of "0–3 h" and "3–6 h" during zero to 24 hours after referral showed an ultimately increasing trend, but in the group of patients with the time of onset of pain> 6 h had an entirely decreasing trend (Figure 2).

Troponin and CMyBP-C in the AMI group were significantly higher at all studied times compared to the control group (P <0.001). The value of AUC of 1.00 (95% CI: 1.00-1.00) indicated that the CMyBP-C marker fully and correctly identified all AMI individuals (Figure 3).At a significance level of 5%, the P-value <0.001 indicated that the CMyBP-C marker has an excellent ability to diagnose disease in the patients.

Table 1. Studied variables during different periods of time in both control and case groups

Group Variables		Control (N = 60)	Case (MI patients) (N = 65)
Age, year, N (%)	18-29	5 (8.3)	1 (1.5)
	30-59	52 (86.7)	38 (58.5)
	≥ 60	3 (5)	26 (40)
Gender, Male, N (%)		32 (53.3)	40 (61.5)
Underlying disease, N (%)	Diabetes	3 (5)	1 (1.5)
	Hyperlipidemia	5 (8.3)	1 (1.5)
	Smoking	14 (23.3)	3 (4.6)
	Blood pressure	4 (6.7)	1 (1.5)
MI, N (%)	STEMI	-	42 (64.6)
	Non-STEMI	-	23 (35.4)
ECG changes, N (%)	Lower leads	-	17 (26.2)
	Side leads	-	8 (12.3)
	Right anterior leads	-	2 (3.1)
	Wide anterior leads	-	16 (24.6)
	New LBBB	-	4 (6.2)
Pain onset time (hours), N (%)	< 3	-	26 (40)
	3-6	-	34 (52.3)
	> 6	-	5 (7.7)

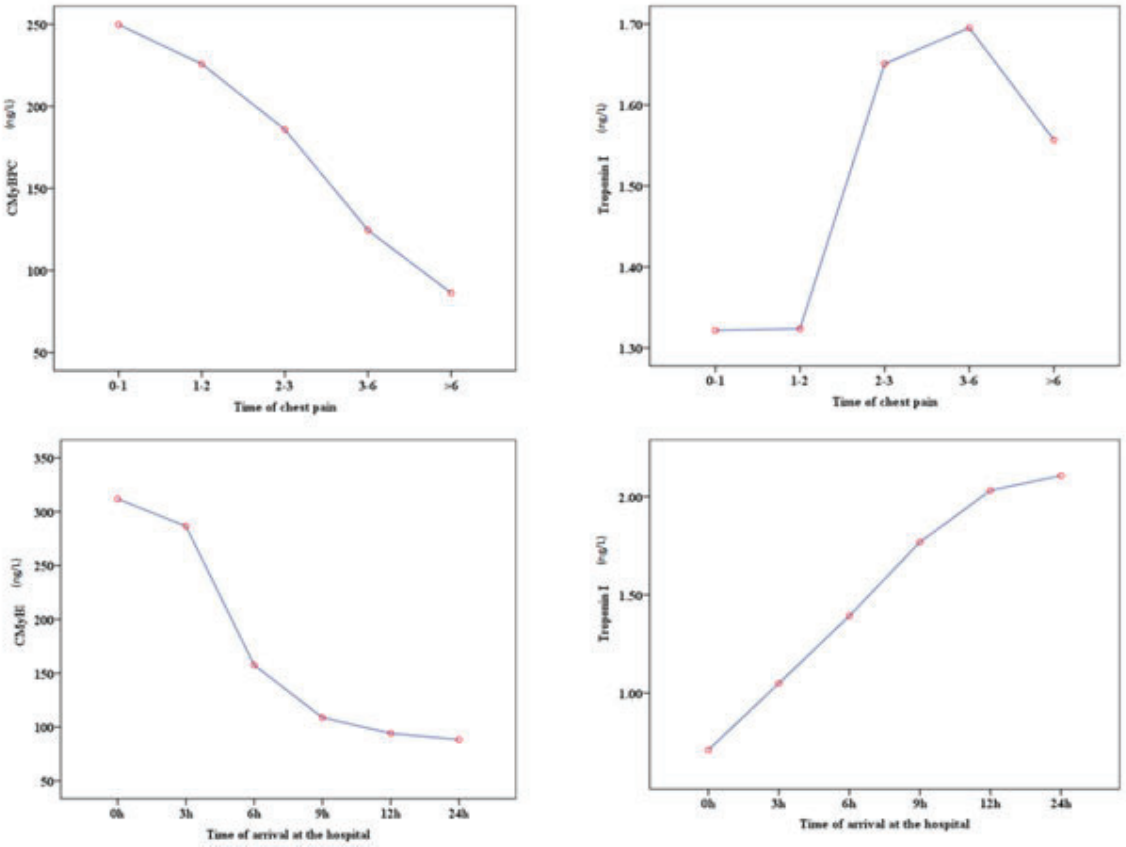

Figure 1. The trend of changes in CMyBPC and troponin levels in the patients since the onset of chest pain, and the time of arrival at the hospital.

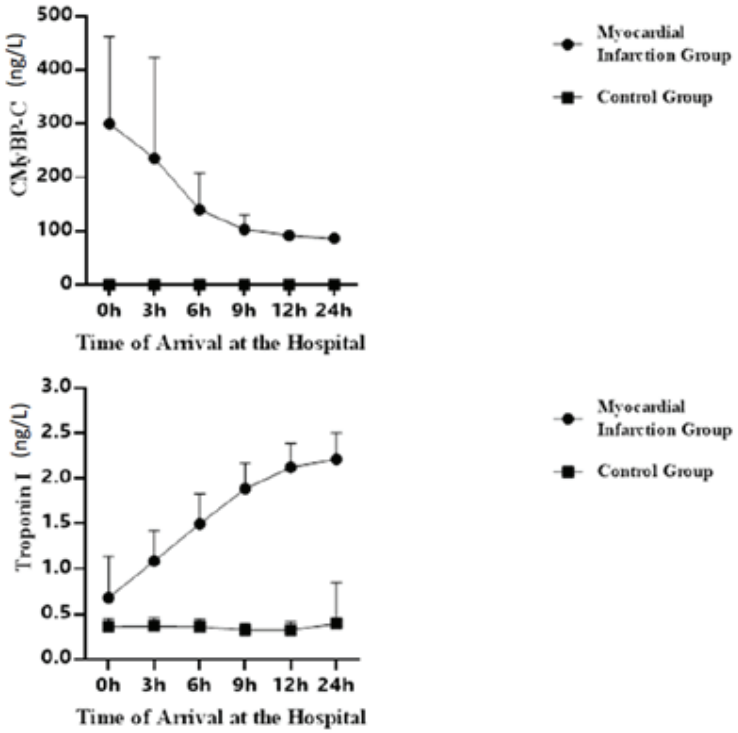

Figure 2. Trend of changes in CMyBPC and troponin at different times.

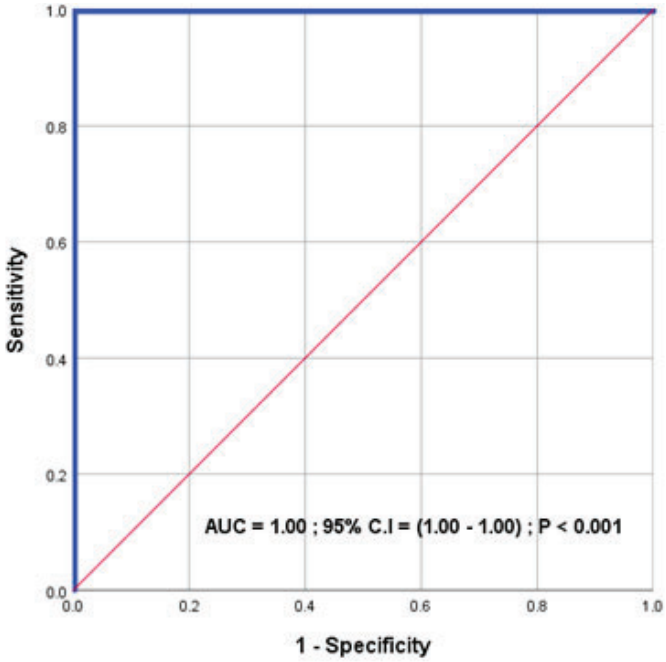

Figure 3. ROC Curve of CMyBP-C biomarker.

DISCUSSION

cMyC is the first cardiac tissue-specific protein to be reported as a diagnostic tool in AMI after cTn. In the present study, we examined changes in cMyC and cTn in AMI patients. Our research findings showed that this biomarker was significantly higher in the group of patients with AMI at all times studied and has high diagnostic accuracy in diagnosing patients with AMI. It was also found that the mean cMyC decreases as time elapses since chest pain or since hospitalisation. Therefore, the diagnostic value of this test is in the early hours.

The cMyC protein was first discovered in 1973 by Afer *et al*. with three isoforms specific for short bones, slow bones, and skeletal muscle. Skeletal muscle isoforms are widely expressed in cardiac tissue from embryonic time. Like cTnT and cTnI, this protein has a specific expression for heart tissue, but its amount is much higher (20,21).

In 2013 a study in the USA by Govindan *et al.,* calculated the trend in serum CmyBP-C levels following AMI and compared these with other available biomarkers (16). In our case-control study, serum CmyBP-C levels were measured by sandwich ELISA and CmyBP-C levels were significantly higher in AMI patients. However, a recent study has shown that CmyBP-C was 257 (75-876) ng/L for Type 1 MI with an AUC of 0.67 (95% CI: 0.61-0.73) (22). But, another study was showed high predictive power (0.967) for cMyBP-C cardiac biomarkesimilar to our results (23).

In another study biomarkers, such as myoglobin, carbonic anhydrase, and creatinine MB were significantly increased in people with MI while levels of cardiac troponin 1, glycogen phosphorylase, and cardiac fatty acids bound to the protein did not change significantly (21). The findings of that study are entirely consistent with the results of our study and showed a significant increase in CmyBP-C levels in patients with AMI. Also, it was found that the diagnostic power of CmyBP-C is high and can detect myocardial infarction with high accuracy in the early hours (21). Also, Diederik *et al.* examined the release kinetics of myosin-binding protein C in a swine model and two human groups. Measurement of CmyBP-C levels was measured 30 minutes to 14 days after coronary occlusion. The plasma level of this protein reached its baseline level (76 positive and negative 68 ng/l) three hours later (767 negative 211 ng/l) and its peak after 6 hours (2.418 positive and negative 780 ng). Per liter) was observed. The level of cMyBP-C wassimilarly reached in humans 4 hours later (12). However, in our study, it was found that the level of CmyBP-C is at its highest level in the very first hours of pain and hospitalisation and decreases over time. This difference in results may be due to sampling, differences in exclusion criteria, differences in control of the effect of confounders, differences in factors affecting CmyBP-C.

In another study patients with AMI, TASH, and CABG were evaluated (20). Serum samples were collected from these patients, and then CmyC was measured. Finally, this measurement was compared with troponin. Their results showed that following a proven myocardial injury, an increase and decrease in serum cMyC levels occurred much faster than cTnT (20). The findings of their study are similar to our study. Our study also found that CmyBP-C levels decreased over time after the onset of MI and may not be as accurate in the following hours, unlike troponin, which increased over time. Therefore, the use of CmyBP-C in the early hours has high diagnostic accuracy in diagnosing AMI.

In another study CmyBP-C levels were measured in heart failure patients at time of referral and one month after treatment. CmyBP-C levels in patients with heart failure (positive: mean 122.44 ng/ml; negative: mean 1.01 ng / ml) at the time of referral were significantly higher than the control group (positive: mean 24.40 ng/ml; negative: mean 9.83 ng/ml). The increase in CmyBP-C was associated with the severity of heart failure according to the Ross classification. In addition, the level of CmyBP-C was significantly associated with echocardiography and clinical evaluation of the heart. Finally, CmyBP-C was a promising biomarker for detecting heart failure with 100% sensitivity and 96% specificity and a 45 ng/ml cut-off (24). Also, Tong *et al.* showed CmyBP-C to be usefulness as a predictor of cardiovascular events with an AUC of 0.91 (25). That study's findings are in line with our results and show a significant increase in CmyBP-C in AMI. The AUC in our study is equal to 1, which is the highest possible level and shows CmyBP -C's high accuracy in diagnosing AMI.

Our study had sample size limitations that suggests using a widening sample size in cardiovascular disease as a predictive indicator. In conclusion, findings of our study showed that the cMyC biomarker was significantly higher in the group of patients with AMI at all studied times and had a high diagnostic accuracy in diagnosing patients with AMI. It was also found that

cMyC levels decreased as time elapses since chest pain or since hospitalisation. Therefore, the diagnostic value of this test is in the early hours. Due to the high accuracy of cMyC in diagnosing AMI, it should be used as a diagnostic test together with other tests to confirm AMI in the early hours.

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AUTHOR INFORMATION

Kambiz Masoumi: MD, Emergency Medicine Specialist and Associate Professor[1]
Arash Forouzan: MD, Emergency Medicine Specialist and Associate Professor[1]
Hassan Motamed: MD, Emergency Medicine Specialist and Associate Professor[1]
Habib Heybar: MD, Cardiology Specialist and Associate Professor[2]
Nastaran Ranjbari: MD, Pathology Specialist and Associate Professor[3]
Sepideh Mohajer Shirazi: MD, Emergency Medicine Specialist[1]

[1]Department of Emergency Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Department of Cardiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of Pathology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Corresponding author: Dr. Hassan Motamed. Email: researchiran5@gmail.com.

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