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Comparison of High-Sensitive Cardiac Troponin-T Changes between Non-ST-Segment Elevation Myocardial Infarction and Non-Coronary Artery Patients

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Abstract

Background: In the absence of acute coronary syndrome (ACS), various conditions may increase the level of cardiac troponins.
Objectives: We aimed to determine the absolute and relative changes of high-sensitive cardiac troponin T (hs-cTnT) in 2 groups of patients: non-ST-segment-elevation myocardial infarction (NSTEMI) and non-ACS patients.
Methods: In this longitudinal study, performed between December 2011 and July 2012, we calculated the absolute and relative changes of hs-cTnT and troponin velocity in consecutive patients, admitted to the emergency department of Tehran Heart Center. The patients had symptoms of acute MI with elevated baseline hs-cTnT levels. Blood sampling was performed 3 times following admission. The hs-cTnT changes were evaluated and compared between the 2 groups of NSTEMI and non-ACS patients.
Results: After exclusion, the second and third samples (taken 12 and 36 hours after admission on average, respectively) were available in 889 and 641 patients, respectively. Cardiovascular risk factors, including hyperlipidemia, diabetes mellitus, smoking, and history of coronary events or intervention, were significantly more frequent in the NSTEMI group, compared to the non-ACS group. The hs-cTnT level at baseline and in the subsequent samples was significantly higher in the NSTEMI group, compared to the non-ACS group. Also, the absolute and relative changes in hs-cTnT were more significant in the NSTEMI group. Moreover, hs-cTnT velocity within the first 12 hours was significantly higher in the NSTEMI group, compared to the non-ACS group (18.85 $\pm$ 84.89 ng/L/h vs. 4.96 $\pm$ 14.58 ng/L/h; $P < 0.001$).
Conclusions: The changes in hs-cTnT (ie, absolute, relative, and velocity changes) were more significant in the NSTEMI group in comparison with non-ACS patients.

Keywords: Acute Coronary Syndrome, Myocardial Infarction, Troponin T

1. Background

Cardiac troponin (cTn) is the gold standard biomarker in detecting cardiac necrosis, especially in acute myocardial ischemia (AMI), which is the most important cause of myocardial necrosis (1). AMI is the main cause of mortality and morbidity worldwide. Therefore, it is critical that rapid and precise diagnosis be made for the initiation of evidence-based management, including early revascularization (2).

According to the current universal definition of AMI, at least 1 cTn value > 99th percentile is required in patients suspicious of AMI. Based on the available literature, drawing blood samples for cTn measurement between 6 and 9 hours after admission can optimize the clinical sensitivity for AMI diagnosis (1). The high-sensitive cardiac troponin T (hs-cTnT) test is a newly introduced measure (3) and is a modification of the fourth-generation cTn assay with a 99th percentile cutoff point of 0.014 ng/mL (10% coefficient of variation, 0.013 ng/mL).

Nevertheless, the consequences of hs-cTnT test application instead of standard cTn have resulted in a rise in the incidence of AMI from 18% to 22% (4). In addition, there are several noncoronary-related conditions, known to cause an increase in cTn, which should be considered in the differential diagnosis of patients presenting with troponin elevation (5-8). Also, there are several factors which can influence the upper reference limits of hs-cTnT (97.5th and 99th percentiles), such as age, renal function, and gender (9).

A rise and/or fall in hs-cTnT level should be regarded as an index for discriminating between acute and chronic cardiomyocyte damage (10). Furthermore, evidence shows that even a minor rise in troponin level can increase the adverse outcomes and the overall mortality, regardless of the

underlying condition, which is either acute coronary syndrome (ACS) or non-ACS (11, 12).

There are limited studies on the kinetic changes of the recently implemented hs-cTnT assay for discriminating AMI patients from other low-risk patients, presenting to the emergency department (1, 2, 10). Since patients with ST-segment-elevation MI (STEMI) have a hallmark of ST-segment elevation in electrocardiography (ECG), the main challenge is to differentiate between patients presenting with non-STEMI (NSTEMI) and those with non-ACS.

By using serial hs-cTnT assays in patients with elevated hs-cTnT, we aimed to investigate whether changes in hs-cTnT, including absolute, relative, and velocity changes (the rate of change over time), are of a higher accuracy and should as such be preferred for a rapid and accurate differentiation between NSTEMI and non-ACS patients in the emergency setting.

2. Methods

2.1. Sample Collection

In this longitudinal study, performed between December 2011 and July 2012, all consecutive patients, admitted to the emergency department of Tehran heart center, a tertiary care teaching hospital, were recruited with symptoms of AMI, such as chest pain and angina pectoris, as well as elevated baseline hs-cTnT level ($\geq$ 14 ng/L).

If a patient had STEMI on ECG or showed more than a 10% drop in sequential hs-cTnT compared to the baseline, he/she was excluded from the study. The study was in accordance with the principles of the declaration of Helsinki and was approved by the institutional review board in November 2011 (code number, 173). Written informed consents were obtained from all the patients. The time intervals were classified into 3 groups: 4 - 12 hours; > 12 hours; and $\leq$ 24 hours and > 24 hours.

2.2. Laboratory Measurements

Elecsys 2010 (Roche Diagnostics) was used to measure hs-cTnT via electrochemiluminescence immunoassay. The interassay variations for determining normal and abnormal hs-cTnT elevations were 5.3% and 2.4%, while the intraassay variations were 3.6% and 2.2%, respectively, with a lower detection limit of 3 ng/L. The cutoff value for hs-cTnT with a reference interval of 99% was 14 ng/L.

2.3. Study Protocol

All the patients underwent an initial clinical assessment, which comprised of clinical history-taking, physical examination, 12-lead ECG, and standard blood tests. In case of dispute, the final diagnosis of each patient was adjudicated by 2 independent cardiologists, who reviewed all the available medical records (including patient history, physical examination, laboratory tests, ECG, echocardiography, and coronary angiography). The kappa coefficient for interobserver variability was 0.87. In case of dispute, the final diagnosis was established by agreement.

The patients were divided into 2 main groups of NSTEMI and non-ACS. NSTEMI was defined according to the third universal definition of MI (13) after the exclusion of STEMI. The subgroups of non-ACS group were as follows: (1) tachy-brady arrhythmia (tachycardia defined as heart rate $\geq$ 140 beats/min and bradycardia, defined as heart rate < 50 beats/min); (2) systolic heart failure (if the patient's left ventricular ejection fraction was < 45% on ECG); (3) pulmonary thromboembolism; (4) renal failure (patients with baseline creatinine level > 2 mg/dL); (5) hypertension acceleration (baseline blood pressure > 180/110 mmHg); (6) sepsis (systemic inflammatory response induced by severe infection); (7) hypertrophic cardiomyopathy (increased interventricular septum > 15 mm without a specific cause); (8) aortic dissection; (9) cor pulmonale; and (10) unknown factors.

Along with the baseline hs-cTnT value, absolute and relative changes were calculated from serial measurements. Troponin velocities were calculated as follows:

[Maximum subsequent hs-cTnT within 12 hours - initial hs-cTnT]/time between the initial and subsequent hs-cTnT measurements

Patients with STEMI or a falling troponin level (hs-cTnT decline to $\geq$ 10% from the baseline in the second or third sample) were excluded.

2.4. Statistical Analysis

The data are expressed as mean $\pm$ SD and median values with interquartile ranges, as appropriate. To compare continuous variables with normal and nonnormal distributions, t test and Mann–Whitney U test were performed. For the comparison of categorical variables, Fisher's exact test and χ^2 test were employed, as appropriate. The relative and absolute values were calculated, as we were interested in the comparison of the magnitude of changes in these 2 values. The reported P-values were 2-sided, and P value $\leq$ 0.05 was considered statistically significant.

3. Results

During the study, a total of 1563 consecutive patients, admitted to the emergency department due to symptoms of ACS and at least 1 positive hs-cTnT sample (hs-cTnT $\geq$ 14 ng/L), were assessed. After exclusion, the second and third

samples, which were taken 12 and 36 hours after admission on average, were available in 889 and 641 patients, respectively (Figure 1).

The final diagnoses in the non-ACS group were as follows: heart failure in 67 patients, arrhythmia in 34 patients, pulmonary embolism in 11 patients, renal failure in 12 patients, hypertension crisis in 17 patients, sepsis in 13 patients, myopericarditis in 11 patients, hypertrophic cardiomyopathy in 1 patient, aortic dissection in 3 patients, cor pulmonale in 3 patients, and unknown diagnosis in 17 patients.

The baseline characteristics of all 889 patients are presented in Table 1. Cardiovascular risk factors, including hyperlipidemia, diabetes mellitus, and smoking, were significantly more frequent in the NSTEMI group in comparison with the non-ACS group. Also, history of coronary events or intervention was significantly more frequent in the NSTEMI group (Table 1). The hs-cTnT level at baseline and in the 2 subsequent samples was significantly higher in the NSTEMI group in comparison with the non-ACS group (Figure 2). In addition, the changes in hs-cTnT (absolute, relative, and velocity changes) were more significant in the NSTEMI group, compared to the non-ACS group (Table 2) and (Figure 3).

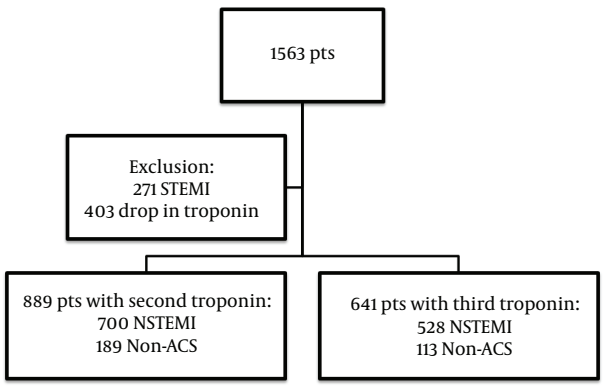

Figure 1. Flowchart of the Study

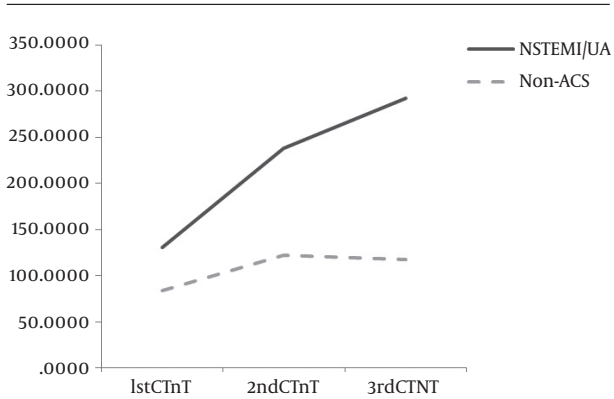

Figure 2. The Mean hs-cTnT Level was Significantly Higher at Baseline and in the Subsequent Samples

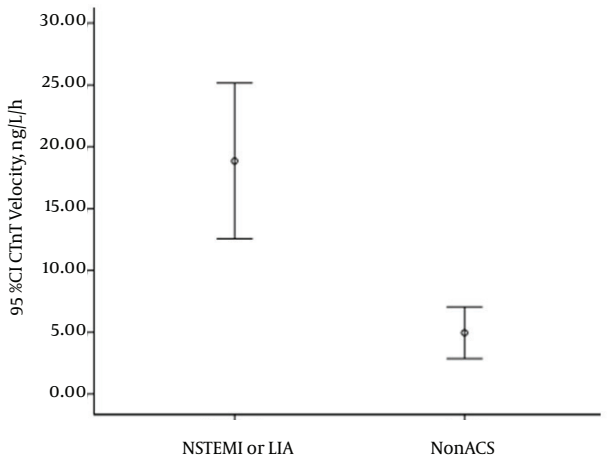

Figure 3. Velocity of hs-cTnT was Higher in the NSTEMI Group

4. Discussion

The current study was performed on 889 consecutive patients with symptoms of AMI and positive hs-cTnT (with the exception of STEMI), presenting to the emergency department. Cardiovascular risk factors, including hyperlipidemia, diabetes mellitus, and smoking, as well as history of coronary events or intervention, were significantly more frequent in the NSTEMI group, compared to the non-ACS group. Also, the mean hs-cTnT and hs-cTnT changes were significantly greater among patients with NSTEMI.

In a study by Reichilin et al. (1), the highest level of hs-cTnT was reported in the ACS group. However, troponin elevation could be also observed in noncoronary conditions, such as heart failure, pulmonary thromboembolism, pulmonary arterial hypertension, stroke, sepsis, acute aortic dissection, acute myopericarditis, hypovolemia, myocardial contusion, Takotsubo cardiomyopathy, and tachycardia; it might even represent a major diagnostic challenge to clinicians (14-19).

The proposed mechanisms for troponin elevation following myocardial damage in noncoronary conditions include a combination of supply/demand imbalance and direct myocardial toxicity. Other underlying mechanisms are abnormal right- and left-ventricular loading, decreased time of diastolic coronary perfusion in tachycardia, stren-

Table 1. Baseline Characteristics of 889 Patients[a]

	NSTEMI (n = 700)	Non-ACS (n = 189)	P Value	All Patients (n = 889)
Age, y	66.8 ± 11.7	66.3 ± 16.8	0.706	66.7 ± 12.9
Male	476 (68.0)	102 (54.0)	< 0.001	578 (65.0)
Risk factors				
Hypertension	426 (60.9)	109 (57.7)	0.427	535 (60.2)
Hyperlipidemia	275 (39.3)	58 (30.7)	0.030	333 (37.5)
Diabetes mellitus	280 (40.0)	48 (25.4)	< 0.001	328 (36.9)
Smoking	169 (24.1)	28 (14.8)	0.006	197 (22.2)
Family history	85 (12.1)	16 (8.5)	0.157	101 (11.4)
History of MI	159 (22.7)	14 (7.4)	< 0.001	173 (19.5)
History of heart failure	110 (15.7)	23 (12.2)	0.225	133 (15.0)
History of PCI	231 (33.0)	8 (4.2)	< 0.001	239 (26.9)
History of CABG	237 (33.9)	21 (11.1)	< 0.001	258 (29.0)
Serum creatinine, mg/dL	1.29 ± 0.93	1.34 ± 1.01	0.361	1.30 ± 0.95

Abbreviations: CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention.
[a]Values are expressed as mean ± SD or No. (%).

Table 2. Changes in hs-CTnT in Patients

	NSTEMI	Non-ACS	P Value
Baseline hs-CTnT	130.73 ± 309.05	83.91 ± 159.72	0.011
Second hs-CTnT	237.92 ± 636.93	122.18 ± 278.59	< 000.1
Third hs-CTnT	292.36 ± 667.34	117.98 ± 253.61	< 000.1
Absolute hs-CTnT changes (0 - 12 h)	107.18 ± 473.82	38.27 ± 141.97	< 000.1
Absolute hs-CTnT changes (12 - 36 h)	75.24 ± 503.41	-10.5 ± 108.10	< 000.1
Relative hs-CTnT changes (0 - 12 h)	1.89 ± 7.26	0.57 ± 2.48	0.007
Relative hs-CTnT changes (12 - 36 h)	1.37 ± 10.02	-0.03 ± 0.33	< 000.1
hs-CTnT velocity, ng/L/h (within 12 h)	18.85 ± 84.89	4.96 ± 14.58	< 0.001

Abbreviations: ACS, acute coronary syndrome; NSTEMI, non-ST-elevation myocardial infarction; hs-CTnT, high-sensitive cardiac troponin T.

uous exercise, catecholamine release, autonomic nervous system imbalance, and impaired membrane permeability.

In heart failure, subendocardial ischemia/necrosis and ongoing apoptosis, induced by myocardial stretch or toxic cytokines, are believed to be the main causes of troponin release (14, 18, 20). Nevertheless, in type-I ACS, coronary atherosclerotic plaque rupture and coronary occlusion constitute the major mechanisms of myocardial damage and troponin elevation (13, 21, 22).

Reichilin et al. (1) reported that absolute changes (Δ) in cTn at 6 hours after admission were superior to relative changes (Δ%) in patients with both low and high baseline cTn levels. They considered the optimal criterion of absolute changes to be about 0.007 μg/L for 2-hour intervals. In contrast, Xu et al. (3) believed that in situations with minor elevations in cTn level, kinetic changes in cTn play an important role in the distinction of coronary artery disease from noncoronary diseases, especially with the use of hs-cTnT assay.

A study by Linder et al. (6) focused on the prevalence of non-ACS putative causes of hs-cTnT elevation but provided no data on the range of changes. Also, Reichlin et al. (1) reported that the area under the receiver operating characteristic (ROC) curve for the diagnosis of AMI was significantly greater for 2-hour absolute versus 2-hour relative cTn changes (AUC 95% CI for hs-cTnT, 0.95 [0.92 to 0.98] vs. 0.76 [0.70 to 0.83]; P < 0.001). Furthermore, in their study, the ROC curve-derived cutoff value for the 2-hour absolute hs-cTnT changes was 7 ng/L.

Additionally, Haaf et al. (2) demonstrated that by adding hs-cTnT changes in the first hour to its presentation value, the diagnostic accuracy for AMI (as quantified by the area under the ROC curve) reached 0.94 for hs-cTnT. In addition, the authors reported that lower cutoff values for relevant absolute changes might be necessary (0.003 - 0.005 μg/L).

In addition to Δ troponin changes, troponin velocity, as a marker of temporal kinetics of troponin eleva-

tion, might differ between myocardial necrosis secondary to plaque rupture (type-I ACS) and supply/demand imbalance or direct myocardial toxicity (the main mechanisms in non-ACS) (23, 24). Troponin velocity might aid in the early risk stratification of patients presenting with unclear reasons for troponin elevation and could present a subset of patients who would potentially benefit from early intervention. In addition, Chuang et al. (23) showed that troponin velocity $\geq$ 2.5 ng/L/h within the first 6 hours was strongly associated with the increased risk of 12-month cardiac-specific and cardiac-related mortality or recurrent MI in patients with or without a final diagnosis of ACS.

The present study showed that hs-cTnT velocity within the first 12 hours was significantly higher in the NSTEMI group in comparison with the non-ACS group (18.85 $\pm$ 84.89 ng/L/h vs. 4.96 $\pm$ 14.58 ng/L/h; $P < 0.001$), similar to absolute and relative hs-cTnT changes. However, more prospective studies with larger sample sizes are needed to better evaluate hs-cTnT velocity. Additionally, further research is required to compare hs-cTnT velocity changes with absolute and relative changes and specify an hs-cTnT velocity cutoff point to better distinguish patients with ACS from non-ACS patients in the emergency setting.

4.1. Conclusions

The present results demonstrated that the baseline values of hs-cTnT and changes in hs-cTnT (absolute, relative, and velocity changes) were significantly higher in patients with NSTEMI in comparison with non-ACS patients.

Footnote

Conflicts of Interest: The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Reichlin T, Irfan A, Twerenbold R, Reiter M, Hochholzer W, Burkhalter H, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. *Circulation.* 2011;**124**(2):136–45. doi: 10.1161/CIRCULATIONAHA.111.023937. [PubMed: 21709058].
2. Haaf P, Drexler B, Reichlin T, Twerenbold R, Reiter M, Meissner J, et al. High-sensitivity cardiac troponin in the distinction of acute myocardial infarction from acute cardiac noncoronary artery disease. *Circulation.* 2012;**126**(1):31–40. doi: 10.1161/CIRCULATIONAHA.112.100867. [PubMed: 22623715].
3. Xu RY, Zhu XF, Yang Y, Ye P. High-sensitive cardiac troponin T. *J Geriatr Cardiol.* 2013;**10**(1):102–9. doi: 10.3969/j.issn.1671-5411.2013.01.015. [PubMed: 23610580].
4. Reichlin T, Twerenbold R, Maushart C, Reiter M, Moehring B, Schaub N, et al. Risk stratification in patients with unstable angina using absolute serial changes of 3 high-sensitive troponin assays. *Am Heart J.* 2013;**165**(3):371–8 e3. doi: 10.1016/j.ahj.2012.11.010. [PubMed: 23453106].
5. Jeremias A, Gibson CM. Narrative review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. *Ann Intern Med.* 2005;**142**(9):786–91. [PubMed: 15867411].
6. Lindner G, Pfortmueller CA, Braun CT, Exadaktylos AK. Non-acute myocardial infarction-related causes of elevated high-sensitive troponin T in the emergency room: a cross-sectional analysis. *Intern Emerg Med.* 2014;**9**(3):335–9. doi: 10.1007/s11739-013-1030-y. [PubMed: 24326466].
7. Kelley WE, Januzzi JL, Christenson RH. Increases of cardiac troponin in conditions other than acute coronary syndrome and heart failure. *Clin Chem.* 2009;**55**(12):2098–112. doi: 10.1373/clinchem.2009.130799. [PubMed: 19815610].
8. Jenab Y, Pourjafari M, Darabi F, Boroumand MA, Zoroufian A, Jalali A. Prevalence and determinants of elevated high-sensitivity cardiac troponin T in hypertrophic cardiomyopathy. *J Cardiol.* 2014;**63**(2):140–4. doi: 10.1016/j.jjcc.2013.07.008. [PubMed: 24011925].
9. Chenevier-Gobeaux C, Bailleul S, Mzabi A, Blanc MC, Lefevre G. Upper Reference Limits of High-Sensitivity Cardiac Troponin T in a General Population: Comparison with those of Sensitive Cardiac Troponin I. *Clin Lab.* 2013;**59**(03+04/2013) doi: 10.7754/Clin.Lab.2012.120230.
10. Twerenbold R, Jaffe A, Reichlin T, Reiter M, Mueller C. High-sensitive troponin T measurements: what do we gain and what are the challenges? *Eur Heart J.* 2012;**33**(5):579–86. doi: 10.1093/eurheartj/ehr492. [PubMed: 22267244].
11. Melki D, Lugnegard J, Alfredsson J, Lind S, Eggers KM, Lindahl B, et al. Implications of Introducing High-Sensitivity Cardiac Troponin T Into Clinical Practice: Data From the SWEDEHEART Registry. *J Am Coll Cardiol.* 2015;**65**(16):1655–64. doi: 10.1016/j.jacc.2015.02.044. [PubMed: 25908071].
12. Giannitsis E, Katus HA. Cardiac troponin level elevations not related to acute coronary syndromes. *Nat Rev Cardiol.* 2013;**10**(11):623–34. doi: 10.1038/nrcardio.2013.129. [PubMed: 23979214].
13. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *Circulation.* 2012;**126**(16):2020–35. doi: 10.1161/CIR.0b013e31826e1058. [PubMed: 22923432].
14. Agewall S, Giannitsis E, Jernberg T, Katus H. Troponin elevation in coronary vs. non-coronary disease. *Eur Heart J.* 2011;**32**(4):404–11. doi: 10.1093/eurheartj/ehq456. [PubMed: 21169615].
15. Jenab Y, Lotfi-Tokaldany M, Alemzadeh-Ansari MJ, Seyyedi SR, Shirani S, Soudaee M, et al. Correlates of syncope in patients with acute pulmonary thromboembolism. *Clin Appl Thromb Hemost.* 2015;**21**(8):772–6. doi: 10.1177/1076029614540037. [PubMed: 24989710].
16. Lankeit M, Friesen D, Aschoff J, Dellas C, Hasenfuss G, Katus H, et al. Highly sensitive troponin T assay in normotensive patients with acute pulmonary embolism. *Eur Heart J.* 2010;**31**(15):1836–44. doi: 10.1093/eurheartj/ehq234. [PubMed: 20584774].
17. Sanati HR, Alemzadeh-Ansari MJ, Zahedmehr A, Azarshab A, Firouzi A, Shakerian F, et al. Evaluation of Cardiac Biomarkers after Percutaneous Balloon Mitral Commissurotomy. *Iran Heart J.* 2016;**17**(1):64–70.
18. Tanindi A, Cemri M. Troponin elevation in conditions other than acute coronary syndromes. *Vasc Health Risk Manag.* 2011;**7**:597–603. doi: 10.2147/VHRM.S24509. [PubMed: 22102783].
19. Jenab Y, Ghaffari-Marandi N. Changes of high-sensitive troponin level in a patient with paroxysmal supraventricular tachycardia. *Anadolu Kardiyol Derg.* 2012;**12**(1):77–8. doi: 10.5152/akd.2012.018. [PubMed: 22231940].
20. Blich M, Sebbag A, Attias J, Aronson D, Markiewicz W. Cardiac troponin I elevation in hospitalized patients without acute coronary syndromes. *Am J Cardiol.* 2008;**101**(10):1384–8. doi: 10.1016/j.amjcard.2008.01.011. [PubMed: 18471446].
21. French JK, White HD. Clinical implications of the new definition of myocardial infarction. *Heart.* 2004;**90**(1):99–106. [PubMed: 14676259].
22. White HD. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? *J Am Coll Cardiol.* 2011;**57**(24):2406–8. doi: 10.1016/j.jacc.2011.01.029. [PubMed: 21658560].

23. Chuang A, Hancock DG, Horsfall M, Alhammad NJ, Zhou J, Cullen L, et al. The predictive value of high sensitivity-troponin velocity within the first 6h of presentation for cardiac outcomes regardless of acute coronary syndrome diagnosis. *Int J Cardiol.* 2016;**204**:106–11. doi: 10.1016/j.ijcard.2015.11.132. [PubMed: 26655552].

24. Reichlin T, Twerenbold R, Wildi K, Rubini Gimenez M, Bergsma N, Haaf P, et al. Prospective validation of a 1-hour algorithm to rule-out and rule-in acute myocardial infarction using a high-sensitivity cardiac troponin T assay. *CMAJ.* 2015;**187**(8):E243–52. doi: 10.1503/cmaj.141349. [PubMed: 25869867].