Int J Clin Exp Pathol 2015;8(9):11206-11211
www.ijcep.com /ISSN:1936-2625/IJCEP0013497

Original Article

Role of E-selectin for diagnosis of myocardial injury in children of age up to 14 years

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Received July 27, 2015; Accepted August 28, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Background: Effects of myocardial injury on E-selectin remain unclear. Thus, we investigated the diagnostic value of E-selectin for myocardial injury in children of no more than 14 years of age, which determined the scoring method of myocardial injury. Methods: In this prospective study, plasma E-selectin, cardiac troponin I (cTnI) and creatine kinase isoenzyme MB (CK-MB) concentrations in pediatric patients with myocardial injury (myocardial injury group, n=85) were measured. The control group comprised 80 patients without myocardial injury, and the case-control study method was selected at the same time. The definition of cardiac injury was based on cTnI and CK-MB (with or possibly without abnormal ECG evidence). Diagnostic value of E-selectin for myocardial injury was determined by analyzing receiver operating characteristic (ROC) curves. Results: The differences between the two groups were of statistical significance (P<0.001). For the 85 patients with myocardial injury, the area under the ROC curve (AUC) value for plasma E-selectin levels was 0.945 with a 95% CI of 0.899-0.991 and the optimal diagnostic cut-off value 29.67 ng/ml (positive likelihood ratio (positive LR=72.5); AUC value for plasma cTnI level was 0.848 with a 95% CI: 0.737-0.960 and the optimal diagnostic cut-off value was 0.155 μg/L (positive LR=12.3); AUC value for plasma CK-MB levels was 0.946 with a 95% CI: 0.903-0.989 and the optimal diagnostic cut-off value 24.26 IU/L (positive LR=72.5). Conclusions: E-selectin is more effective than cTnI in diagnosing myocardial injury as an important biological marker of myocardial injury- an important index of pediatric cardiac injury score.

Keywords: E-selectin, myocardial injury, diagnostic value, score, children

Introduction

Myocardial injury is complications of many pediatric diseases. Currently, there are no confirmed diagnostic criteria and scoring method for myocardial injury in this condition. Diagnosing myocardial injury is mainly based on myocardium-related protein and myocardial enzymes [2], of which the most cTnI and CK-MB application widely, cTnI are most commonly used, however, cTnI and CK-MB all have defects, and is not an ideal biological markers, thus, alternatives need to be developed. The study shown that E-selectin is detected early and persistent expression when myocardial ischemia injury [1], Therefore, we continuous observed 85 patients with myocardial injury, to understand the expression of E-select levels and the relationship with myocardial injury, and explore the scoring method of myocardial injury in children of age up to 14 years. Optimal cut-off value of E-selectin for myocardial injury was determined by analyzing the area under the curve (AUC), sensitivity, specificity, Youden index and positive likelihood ratio (LR) of receiver operating characteristic (ROC) curves.

Materials and methods

Patients

All subjects were recruited from patients treated at the Affiliated Shunde Women and Children's Hospital of Jinan University between March 2013 and May 2015.

A total of 85 consecutive pediatric patients with myocardial injury were prospectively enrolled based on inclusion and exclusion criteria.

Inclusion criteria: pediatric patients with myocardial injury Exclusion criteria: patients treated for myocardial injury prior to hospital admission.

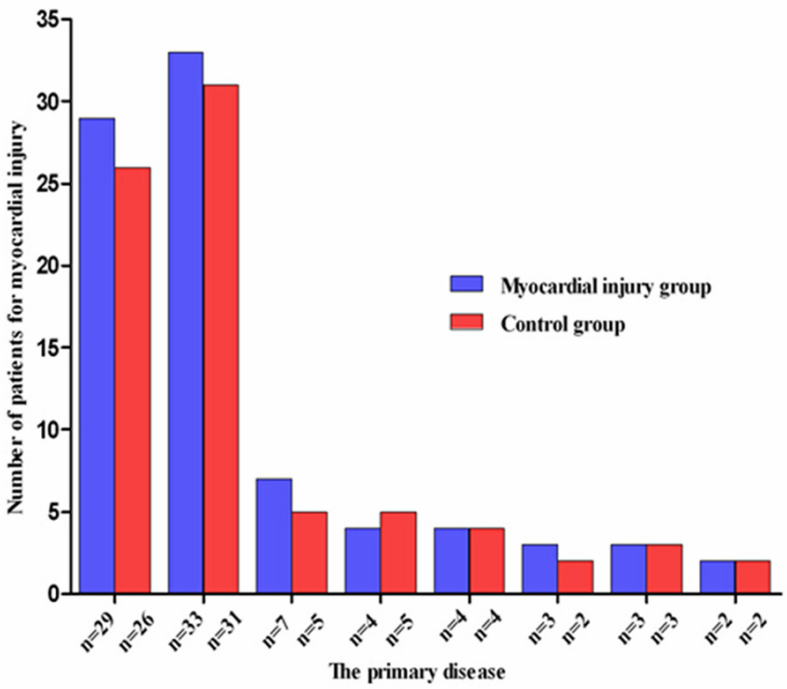

Figure 1. Distribution of primary disease in the myocardial injury patients and no-myocardial injury patients.

Myocardial injury only used cTnI or CK-MB results to make the diagnosis [2]. Indexing reference value: cTnI >0.15 μg/L was myocardial injury, >0.5 μg/L was Myocardial infarction; CK-MB >24 IU/L was myocardial injury [2].

This study was approved by the Ethics Committee of the Affiliated Shunde Women and Children's Hospital of Jinan University. All parents provided written informed consent because the study required a small quantity of extra blood to be collected to carry out the extra assays.

85 consecutive patients with myocardial injury (myocardial injury group) were recruited in the study including 44 males and 41 females, and average age was 2.6 years (range: 0-14 years), 80 patients with case-control study method to select no myocardial injury of children during this period (control group) were recruited in the study including 42 males and 38 females; and average age was 2.5 years (range: 0-14 years). Gender and age distribution similar between the two groups had no statistical significance (P>0.05).

In the myocardial injury patients and no-myocardial injury patients the primary disease included respectively, mycoplasma pneumoniae pneumonia (n=29, n=33), rotavirus enteritis (n=33, n=31), suffocating (n=7, n=5), hand-foot and mouth disease (n=4, n=5), congenital heart disease (n=4, n=4), food poisoning (n=3, n=2), nephrotic syndrome (n=3, n=3), kawasaki disease (n=2, n=2) (**Figure 1**).

Laboratory assays

The study was prospective and in blind manner. Measurements of E-selectin, cTnI and CK-MB levels were fully automated. Venous blood samples (5 ml) were collected in tubes containing EDTA-K2. Blood samples were drawn from subjects (myocardial injury and non myocardial injury groups) during hospital admission.

Plasma E-selectin concentration was assayed by ELISA assay using a ThermoMK3 Enzyme-linked immunity analyzer (Bender Medsystems, Vienna, Austria). Coefficient of variance (CV) within each group was 2.85%, and CV between groups was 3.26%. Currently, there is no defined E-selectin reference range.

Plasma cTnI and CK-MB concentration was analyzed by chemiluminescence using a Beckman Chemiluminescence analyzer (Beckman Coulter, Inc, Kraemer, USA), normal reference range, 0-0.03 μg/L; where >0.15 μg/L is consistent with myocardial injury, and >0.5 μg/L is consistent with myocardial infarction.

Strictly in accordance with the manual operation.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 statistical software (SPSS, Inc., Chicago, IL, USA); and ROC curves were drawn using GraphPad software. (GraphPad Software, Inc. La Jolla, USA).

Table 1. Comparison of E-selectin, cTnI and CK-MB in the myocardia injury patients, non-myocardia injury patients $\bar{X} \pm s$

Subjects (patients)	n	E-selectin	cTnI (μg/L)	CK-MB (IU/L)
Myocardia injury	85	42.25±4.82	0.26±0.02	27.66±4.22
non-myocardia injury	80	12.46±3.29	0.02±0.01	9.46±4.77
t		5.41	9. 87	5.04

Note: E-selectin, cTnI and CK-MB: $P<0.001$.

Statistical analysis was used to determine plasma E-selectin cut-off values for myocardial injury in pediatric patients, which included AUC, 95% CI, sensitivity, specificity, and positive LR. ROC curve statistical analysis was performed to determine the strength and effectiveness of E-selectin, cTnI and CK-MB for diagnosing myocardial injury.

Distributions were tested for normality. For normally-distributed data, continuous variables were tested by an independent sample *t*-test and Mann-Whitney *U*-test. For non-normally distributed data, continuous variables were tested nonparametrically by Wilcoxon test.

Results

Plasma E-selectin, cTnI and CK-MB levels were normally distributed ($P<0.001$); therefore, mean ± standard deviation ($\bar{X} \pm SD$) was used to express results.

Clinical picture

22 cases of breathing difficulties (n=31), tired (n=75), Tachycardia (n=51), bradycardia (n=15) and normal heartbeat (n=19). 65 patients of myocardial injury in children all have abnormal ECG, which show were alone or cross T wave and ST segment abnormality.

Myocardial injury

The positive rate of expression for CK-MB and cTnI levels were 57.64% (n=49) and 42.35% (n=36), respectively in the 85 patients with myocardial injury.

Biomarker data

In the patients with myocardial injury, plasma E-selectin, cTnI and CK-MB levels were 42.25 ±4.82 ng/ml, 0.26±0.02 μg/L and 27.66±4.22 (IU/L), respectively; Results for patients without myocardial injury were 12.46±3.29 ng/ml, 0.02±0.01 μg/L, 9.46±4.77 pg/ml, respectively. There were significant differences between groups for E-selectin, cTnI and CK-MB (*t* =5.41, 9.87 and 5.04; *All P*< 0.001). (**Table 1** and **Figure 2**).

For myocardial injury diagnosis, AUC of E-selectin and cTnI were 0.945 (95% CI: 0.899-0.991), 0.848 (95% CI: 0.737-0.960) and 0.946 (0.903-0.989), respectively. Thus, E-selectin is a better biomarker. Further, sensitivity, specificity and Youden index results for diagnosing myocardial injury by E-selectin were 0.801, 0.999 and 0.801, respectively; while results for diagnosing myocardial injury by cTnI were 0.778, 0.999 and 0.778, respectively; results for diagnosing myocardial injury by CK-MB were 0801, 0.999 and 0.800, respectively. For myocardial injury diagnosis, optimal cut-off values for E-selectin, cTnI and CK-MB were 29.67 ng/ml, 0.155 μg/L and 24.50 IU/L, respectively; while positive LR for E-selectin, cTnI and CK-MB were 72.5, 12.3 and 72.5, respectively (LR >10 have diagnostic value). These results indicate that both analyses were effective for diagnosing myocardial injury (**Table 2** and **Figure 3**).

Optimal cut-off value for plasma E-selectin for diagnosing myocardial injury in pediatric patients was 29.67 ng/ml.

On the basis of above research results, we tentatively proposed the scoring method for myocardial injury in children age up to 14 years, to be used for the diagnosis of pediatric cardiac injury (**Table 3**).

Discussion

Myocardial injury is the complications of many disease [3, 4], the research showed that the myocardial injury is associated with the immune function change of inflammatory mediators caused [4-6]. E-selectin is a member of the selectin family of adhesion molecules and an important inflammatory medium that affects cellular immune functions. It is a cell surface glycoprotein (molecular weight: 107-115 kDa) that binds to active endothelial cell surfaces. When interleukins and TNF-α are released by damaged cells, E-selectin binds to endothelial cells, allowing neutrophil attachment to the vessel wall and consequent aggregation; thus, promoting inflammation and immune injury.

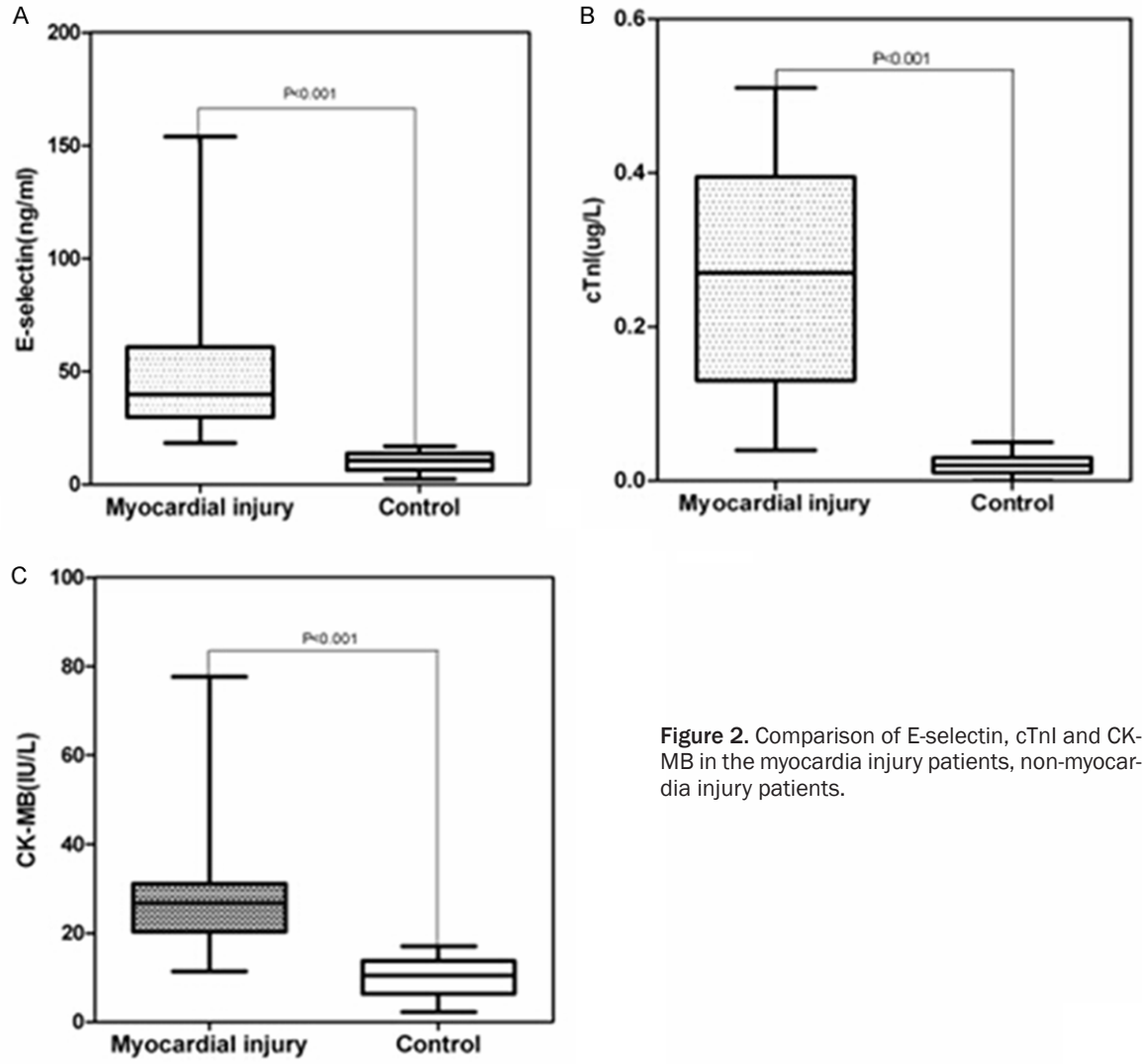

Figure 2. Comparison of E-selectin, cTnI and CK-MB in the myocardia injury patients, non-myocardia injury patients.

Table 2. The cut-off value of plasma E-selectin, cTnI and CK-MB for myocardial injury in pediatric patients

Project	AUC (95% CI)	Sensitivity	Specificity	Youden index	Positive LR	Optimal cutoff
E-selectin	0.945 (0.899-0.991)	0.801	0.999	0.801	72.5	29.67 ng/ml
cTnI	0.848 (0.737-0.960)	0.778	0.999	0.778	12.3	0.155 μg/L
CK-MB	0.946 (0.903-0.989)	0.801	0.999	0.800	72.5	24.50 IU/L

E-selectin overexpression could then be analyzed in plasma. E-selectin expression remains elevated and can be assayed following myocardial ischemia/reperfusion injury [7, 8]. This means that E-selectin is a 'memory' biomarker for myocardial ischemia/reperfusion. Study shows that E-selectin levels have been associated with various cardiovascular diseases [1, 9]. This study confirms previous reports; wherein, E-selectin is unusually high in patients with myocardial injury.

Many biomarkers are used in clinical practice to identify myocardial injury; wherein, cTnI and CK-MB is probably the most commonly used biomarker [4, 10, 11]. However, no marker is perfect, which expression of cTnI and CK-MB are influenced by many factors. Research showed that, the positive rate of CK-MB and cTnI expression about 35-68% and 25-42% respectively when myocardial injury [12, 13], similar to results of this study (57.64% and 42.35%), showed that cTnI and CK-MB positive

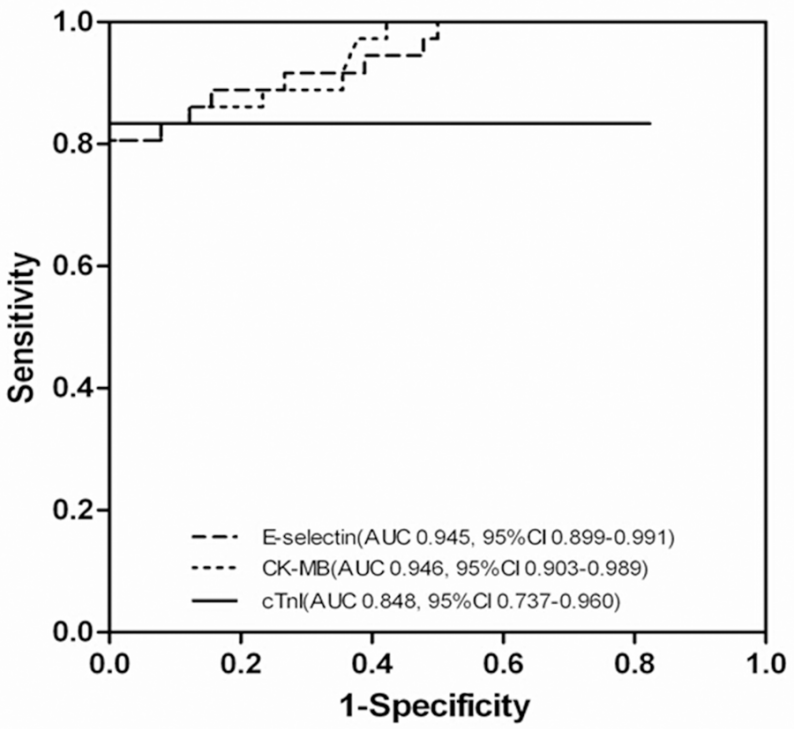

Figure 3. ROC curve of diagnosis value of *E-selectin*, cTnI and CK-MB for myocardial injury.

Table 3. Pediatric cardiac injury score (0-14 years)

	0	1	2	3
Breathing (RR/min)				
0-1 Year	<50	50-60	>60	>80
1-3 Years	<40	40-50	>50	>60
4-7 Years	<30	30-40	>40	>50
8-14 Years	<25	25-30	>30	>40
Weary	no	activity	quietness	extreme
ST-T abnormal (ECG)	no	single lead	2 leads	>3 leads
cTnI (μg/L)	0-0.15	>0.15	>0.3	>0.5
CK-MB (IU/L)	<24	>24	>50	>100
E-selectin (ng/ml)	0-30	>30	>60	>120

Score of myocardial injury: no (0-3), Mild (4-6), moderate (7-9), severe (≥10).

expression of incompleteness when myocardial injury. This is not appropriate that only basis on cTnI or CK-MB index to diagnose myocardial injury. When myocardial injury except cTnI or CK-MB may be unusually elevated, well have difficulty breathing, weary and ST-T (ECG) change [14, 15]. All of these suggest that myocardial injury must have other higher accuracy index and combined with clinical characteristics to confirm.

So far, there is no the diagnostic criteria of myocardial injury, which lack the perfect biomarkers of myocardial injury. Thus, there is a continuous search for better biomarkers. This study shows that AUC of E-selectin is larger, and had greater sensitivity and specificity compared to other biomarkers. The optimal diagnostic cut-off value for myocardial injury was 29.67 ng/ml and positive LR was 72.5, which strongly suggests that the higher accuracy for diagnosis of myocardial injury. Therefore, E-selectin is a very effective new biomarker for myocardial injury.

E-selectin can remain stable for a long time in blood. ELISA is a simple and low cost method that could relatively provide results rapidly (results are available within four hours) with high sensitivity and specificity.

This study has found a new biological marker for myocardial injury, which tentatively proposed the scoring method for myocardial injury in children age up to 14 years. This is important to the diagnosis of pediatric cardiac injury.

Acknowledgements

This study was supported by the Bureau of Science and Technology of Foshan City, China (Grant No. 201308230).

Disclosure of conflict of interest

None.

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References

[1] Hyvelin JM, Tardy I, Bettinger T, von Wronski M, Costa M, Emmel P, Colevret D, Bussat P, Lassus A, Botteron C, Nunn A, Frinking P and Tranquart F. Ultrasound molecular imaging of transient acute myocardial ischemia with a clinically translatable P- and E-selectin targeted contrast agent: correlation with the expression of selectins. Invest Radiol 2014; 49: 224-235.

[2] Alpert JS, Thygesen K, Antman E and Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000; 36: 959-969.

[3] Borkhsenius SN, Chernova OA and Chernov VM. [Interaction of Mycoplasma with immune system of animals and humans]. Tsitologiia 2001; 43: 219-243.

[4] Liu X, Wu Y, Zhu J, Lv X, Tang Y, Sun J and Zhang S. Totally thoracoscopic repair of atrial septal defect reduces systemic inflammatory reaction and myocardial damage in initial patients. Eur J Med Res 2014; 19: 13.

[5] Carrabba N, Valenti R, Migliorini A, Vergara R, Parodi G and Antoniucci D. Prognostic value of myocardial injury following transcatheter aortic valve implantation. Am J Cardiol 2013; 111: 1475-1481.

[6] Hu Q, Chen J, Jiang C and Liu HF. Effect of peroxisome proliferator-activated receptor gamma agonist on heart of rabbits with acute myocardial ischemia/reperfusion injury. Asian Pac J Trop Med 2014; 7: 271-5.

[7] Karakas M, Baumert J, Kleber ME, Thorand B, Dallmeier D, Silbernagel G, Grammer TB, Rottbauer W, Meisinger C, Illig T, März W and Koenig W. A variant in the ABO gene explains the variation in soluble E-selectin levels-results from dense genotyping in two independent populations. PLoS One 2012; 7: e51441.

[8] Yago T, Shao B, Miner JJ, Yao L, Klopocki AG, Maeda K, Coggeshall KM and McEver RP. E-selectin engages PSGL-1 and CD44 through a common signaling pathway to induce integrin alphaLbeta2-mediated slow leukocyte rolling. Blood 2010; 116: 485-494.

[9] Leng X, Wang J, Carson A, Chen X, Fu H, Ottoboni S, Wagner WR and Villanueva FS. Ultrasound detection of myocardial ischemic memory using an E-selectin targeting peptide amenable to human application. Mol Imaging 2014; 13: 1-9.

[10] Hadi NR, Yusif FG, Yousif M and Jaen KK. Both castration and goserelin acetate ameliorate myocardial ischemia reperfusion injury and apoptosis in male rats. ISRN Pharmacol 2014; 10: 206951.

[11] Hobkirk JP, Damy T, WaltersM, Bennett A, Smith SJ, Ingle L, Clark AL and Cleland JG. Effects of reducing inspired Oxygen concentration for one hour in patients with chronic heart failure: implications for air travel. Eur J Heart Fail 2013; 77: 283-289.

[12] Hobkirk JP, Damy T, WaltersM, Bennett A, Smith SJ, Ingle L, Clark AL and Cleland JG. Effects of reducing inspired Oxygen concentration for one hour in patients with chronic heart failure: implications for air travel. Eur J Heart Fail 2013; 77: 283-289.

[13] Li X, Luo R, Jiang R, Kong H, Tang Y, Shu Y and Hua W. The prognostic use of serum concentrations of cardiac troponin-I, CK-MB and myoglobin in patients with idiopathic dilated cardiomyopathy. Heart Lung 2014; 43: 219-224.

[14] Sedaghat-Hamedani F, Zitron E, Kayvanpour E, Lorenz HM, Katus HA and Meder B. Post cardiac injury syndrome after initially uncomplicated CRT-D implantation: a case report and a systematic review. Clin Res Cardiol 2014; 103: 781-789.

[15] Mierzewska-Schmidt M and Gawecka A. Neurogenic stunned myocardium-do we consider this diagnosis in patients with acute central nervous system injury and acute heart failure? Anaesthesiol Intensive Ther 2015; 47: 175-180.