Cardiac Biomarkers after reperfusion injury in open heart surgery

Rasha Nabil Saad Salamah[1], Salah Khalaf[2], Mohammed Mohammed Al Arman[1], Nawal Abd El Gileel[1*]
[1]Department of Clinical Pathology, Mansoura Faculty of Medicine
[2]Department of cardiothoracic surgery, Mansoura Faculty of Medicine
***Corresponding author:** Rasha Nabil Saad Salamah
Mobile: 0097455449005
E mail: rasha.nabil.salama@gmail.com

ABSTRACT

Background: Changes in the electrocardiogram (ECG) and an increase in the release of biochemical markers are used to diagnose perioperative myocardial infarction. Candidates with high levels of troponin T and creatine kinase (CK)-MB have been found to be particularly promising. Unfortunately, CK activity and (CK-MB) are not entirely reliable in terms of their sensitivity and specificity.

Aim & objectives: to assess the effect of reperfusion injury on the cardiac function as assessed by LDH, CK, CK-MB, troponin I, in addition to ECG and ECHO.

Patients and Methods: The research was prospective, meaning that it looked into the future. conducted on Cardiothoracic Department, Mansoura University Hospitals. The study comprised 23 patients subjected to open heart surgery and were separated into two groups: Group I (Ventricular septal defect group) and Group II (Valve replacement group).

Results: there was a statistically significant difference amongst the 2 groups regarding to One way Anova for mean LDH levels, total CK levels, CK-MB levels, Troponin I levels and postoperative ischaemic changes via ECG. There was no statistically significant difference among the 2 groups regarding to Comparison of LDH levels at different times and Correlation between LDH, total CK, CK-MB and Troponin I levels at different times with both ischaemic and perfusion times.

Conclusion: The use of laboratory panels for assessment of an organ function is advocated by many clinicians. Laboratory markers of cardiac damage are now widely used, especially when false negative ECG changes are taken into consideration.

Key words: Cardiac Biomarkers, reperfusion injury, open heart surgery.

Introduction

Ischemia-reperfusion injury is defined as the harm done to cells upon reperfusion into ischemic tissues that were previously functioning normally. 1 Several surgical circumstances, such as organ infarction, transplantation failure, peripheral vascular insufficiency, and infection, and intraoperative methods, such as aortic cross-clamping and cardiopulmonary bypass, contribute to the pathophysiology of ischemia reperfusion damage. Reperfusion causes even more damage to tissues than ischaemia alone does, and this is the key trait. 2

There are two components to a decreased blood flow. One problem is that the myocardium doesn't get enough oxygen and metabolic substrates. Second, washing is limited, leading to buildup of reactive oxygen species (ROS) and metabolites. 3

Superoxide anion radical (O2-), hydrogen peroxide (H2O2), singlet oxygen (O2), and the hydroxyl radical are the most frequent reactive oxygen species (ROS) (HO-). The antioxidant system generally removes ROS. 4,5 In pathological states, an excessive amount of reactive

oxygen species (ROS) are produced, and the antioxidant system is unable to deal with them, leading to cellular damage. An increase in reactive oxygen species (ROS) generation occurs after reperfusion, which contributes to tissue damage. 6

ROS can also be produced by neutrophils. Reoxygenation injury does not necessitate, however, that neutrophils be in an active state. 7, 8

Changes in the electrocardiogram (ECG) and increased release of biochemical markers are currently used to diagnose perioperative myocardial infarction. 9.10

Candidates with high levels of troponin T and creatine kinase (CK)-MB have been found to be particularly promising. Unfortunately, CK activity and (CK-MB) are not entirely reliable in terms of their sensitivity and specificity. 10 Cardiac-specific isoforms exist only for troponins T and I.

Although troponin T was first identified, cTnI has been shown to be as as, if not more, cardio-specific than troponin T, and is undetectable in the sera of healthy volunteers. As a result, its detection in the blood would serve as an unmistakable indicator of heart muscle cell injury. False positive elevations of total LDH are seen in various disorders, therefore while it is sensitive for myocardial injury, it is not specific. 13

Subjects and Methods

This prospective study was designed to assess the myocardial function after open heart surgery. This study comprised 23 patients subjected to open heart surgery and were separated into two groups:

Group I (Ventricular septal defect group): It included 8 patients subjected to ventricular septal defect repair. They were 4 men & 4 women, their ages extended from 1 to 16 years with a mean age of (6.1 ± 5.8 years).

Group II (Valve replacement group): It included 15 patients subjected to valve replacement. They were 5 males & 10 females, their ages ranged from 20 to 63 with a mean age of (31.4 ± 19.6 years).

All patients had their operations performed at the Cardiothoracic Department, Mansoura University Hospitals.

The Exclusion criteria were: Heart bypass surgeries performed in conjunction with other types of cardiac surgery, Subjects above the age of 75, Those who present with preexisting renal and/or hepatic impairment, Use of fibrinolytics within 48 hours of surgery, Disorders of severe coagulation, High blood pressure, high cholesterol, and diabetes, and severe muscular disease or intense activity just before surgery.

To all subjects, the following parameters were carried out: Preoperative assessment (Full clinical examination, Clinical investigations and Laboratory investigations) and Operative and postoperative assessment (Routine laboratory investigations and Special tests).

Statistical Analysis

Excel (Office 2000) and SPSS (Statistical Program for the Social Sciences) were used for statistical analysis on IBM-compatible PCs running Windows 98.

Mann-Whitney test:

By testing the Normality of the data by using K-S test (Kolonogrove-Smirnove test) which revealed to be non-normally distributed. So Mann-Whitney test was used to compare the median values amongst the 2 groups for different enzyme levels at different timings.

Anova test: For comparing qualitative data between more than two groups.

Anova test was used to compare marker levels over time.

Bivariate Correlation: Done to see the relation between the different times of the enzymes and their relation to ischaemic and perfusion times.
ROC curve (reciever operating characteristic curve): For testing sensitivity and specificity for the enzyme levels. The ROC curve was drawn to match the performance of the biochemical diagnostic methods of PMI & to calculate the optimum cut-point which has the highest sensitivity and specificity for the different cardiac markers.
X^2: Used to test non-numeric variables and to match frequencies between subgroups.

Results

Table (1): Preoperative Data of patients of both groups

Data		**Group 1** (N=8)	**Group 2** (N=15)
Age (years)		6.1±5.8	31.4 ± 19.6
Sex (F/M)	M	4/8 (50%)	10/15 (66.7%)
	F	4/8 (50%)	5/15 (33.3%)
E. F (%)		66.12 %	68.7%
ECG			
• **H.R** (b/min) (mean)		100	83.6
• **Rhythm**		8/8 (100 %)	10/15 (66.7 %)
- Sinus		0/8	5/15 (33.3 %)
- Irregular		0/8	5/15 (33.3 %)
• **Arrythmias (A.F)**			
• **Ischaemic time** (in mins) (mean)		49.3	54.1
• **Perfusion time** (in mins) (mean)		76.1	92.4
• **Reperfusion time** (in mins) (mean)		15.3	16
Recovery			
• **D.C shocks**		1/8 (12.5 %)	5/15 (33.3 %)
• **Spontaneous**		7/8 (87.5 %)	10/15 (66.7 %)

Table (2): Postoperative Data of patients of both groups.

	Group 1 (N=8)	**Group 2** (N=15)
Postoperative ECG:		
• H.R (b/min) (mean)	103.5	90.3
• Rhythm:		
- Sinus	8/8 (100%)	10/15 (66.7%)
- Irregular	0/8	5/15 (33.3%)
• Ischaemic changes	8/8 (100%)	2/15 (13.3%)

Arrhythmias (A. F)	0	5/15 (33.3%)

Table (3): One way Anova for mean LDH levels at different times in the studied groups

LDH **Group**	**Baseline U/L**	**2 hrs**	**6 hrs**	**20 hrs**	**P**
		After Aortic cross-clamp release			
Group 1 (N=8)	510.5	828	929.5	1070	**0.003***
Group 2 (N=15)	423.2	718.4	788.5	887.8	**0.004***

P is significant if ≤ 0.05

The table showed that LDH level rises significantly in the following samples at 2 hrs, 6 hrs and 20 hrs after clamp release after reperfusion for group 1 (p=0.003) & group 2 (p=0.004).

Table (4): Comparison of total CK levels at different times in the studied groups.

CK total **Group**		**Baseline U/L**	**2 hrs**	**6 hrs**	**20 hrs**
			After Aortic cross-clamp release		
Group 1 (N=8)	**Median**	70.5	664.5	885.5	703.5
	Minimum	38	223	404	289
	Maximum	310	971	1302	2599
	S.E	32.3	105.1	102.9	287.6
Group 2 (N=15)	**Median**	118	350	400	364
	Minimum	16	127	228	168
	Maximum	740	747	1602	2959
	S.E	44.5	40.9	85.4	175.3
P		0.439	**0.008***	**0.003***	0.121

Mann-Whitney test

P is significant if ≤ 0.05

The table showed that the baseline value of total CK is not significantly different amid the 2 groups but higher than the cut off value. There is a significant difference in the CK level between both groups at 2hrs (p=0.008) and 6 hrs (p=0.003) after clamp release followed by an insignificant difference amongst both groups at 20 hrs after clamp release.

Table (5): One way Anova for mean total CK levels at different times in the studied groups

CK total **Group**	**Baseline U/L**	**2 hrs**	**6 hrs**	**20 hrs**	**P**
		After Aortic cross-clamp release			

Group 1 (N=8)	106.8	658.2	920	1059.1	**0.008***
Group 2 (N=15)	153.6	337.8	490.2	615.5	**0.004***

P is significant if ≤ 0.05

The table Showed that total CK level rises significantly in the following samples at 2 hrs, 6 hrs and 20 hrs after clamp release after reperfusion for group 1 (p=0.008) and group 2 (p=0.004).

Table (6): One way Anova for mean CK-MB levels at different times in the studied groups.

CK-MB **Group**	**Baseline U/L**	**2 hrs**	**6 hrs**	**20 hrs**	**P**
		After Aortic cross-clamp release			
Group 1 (N=8)	12	106.5	78.1	36.3	**0.001***
Group 2 (N=15)	11.6	56.1	52.4	35.6	**0.002***

P is significant if ≤ 0.05

The table Showed that CK-MB level rises significantly in the following sample at 2 hrs after clamp release after reperfusion for group 1 (p=0.001) and group 2 (p=0.002) then declines in the following samples at 6 hrs and 20 hrs after clamp release.

Table (7): Comparison of Troponin I levels at different times in the studied groups:

Group	**Troponin I**	**Baseline ng/ml**	**2 hrs**	**6 hrs**	**20 hrs**
			After Aortic cross-clamp release		
Group1 (N=8)	**Median**	1.18	8.7	5.6	2.3
	Minimum	0.32	3.7	4.4	1.4
	Maximum	2.08	22.9	21.3	13.19
	S.E	0.23	2.27	2.1	1.6
Group2 (N=15)	**Median**	1	1.8	2.4	2.5
	Minimum	0.12	0.41	0.51	1.25
	Maximum	1.5	6.48	9.1	10.4
	S.E	0.10	0.48	0.59	0.59
P		0.332	**0.001***	**0.004***	0.897

Mann-Whitney test
P is significant if ≤ 0.05

The table Showed that the baseline value of Troponin I is not significantly different among the 2 groups but within the reference value. There is a significant difference in Troponin I level amongst both groups at 2 hrs (p=0.001) and 6 hrs (p=0.004) after clamp release followed by an insignificant difference between both groups at 20 hrs after clamp release.

Table (8): Comparison between postoperative ischaemic changes via ECG for each group:

	Postoperative changes		**X^2**	**P**
	Present	**Absent**		
Group 1 (N=8)	8	0	15.9	**0.001***
Group 2 (N=15)	2	13		

Chi-square test used for the determination of significance. P is significant if ≤ 0.05

The table showed that eight out of eight patients developed postoperative ischaemic changes in their ECG in group 1, while two out of fifteen patients developed postoperative ischaemic changes in their ECG in group 2. This shows a high significance between both groups (p=0.001).

Table (9): Correlation between LDH, total CK, CK-MB and Troponin I levels at different times with both ischaemic and perfusion times.

Data		**Ischaemic time**				**Perfusion time**			
		Group 1 (N=8)		**Group 2 (N=15)**		**Group 1 (N=8)**		**Group 2 (N=15)**	
		r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
LDH	**2 hrs**	0.184	0.662	0.235	0.4	0.003	0.995	0.218	0.435
	6 hrs	0.601	0.115	0.117	0.678	0.457	0.255	0.095	0.735
	20 hrs	0.616	0.104	0.246	0.377	0.710	**0.048***	0.417	0.122
CK	**2 hrs**	0.713	**0.047***	0.066	0.816	0.53	0.177	0.094	0.738
	6 hrs	0.055	0.896	0.116	0.553	0.075	0.859	0.195	0.485

	20 hrs	0.422	0.298	0.011	0.97	0.413	0.309	0.385	0.156
CK-MB	**2 hrs**	0.441	0.274	0.193	0.491	0.195	0.644	0.18	0.521
	6 hrs	0.614	0.106	0.248	0.372	0.338	0.413	0.458	0.086
	20 hrs	0.652	0.08	0.086	0.759	0.503	0.204	0.388	0.153
Troponin I	**2 hrs**	0.709	**0.049***	0.052	0.855	0.195	0.644	0.180	0.521
	6 hrs	0.731	**0.040***	0.117	0.677	0.378	0.413	0.458	0.086
	20 hrs	0.706	**0.018***	0.029	0.918	0.503	0.204	0.388	0.153

P is significant if ≤ 0.05.

The table showed that there is a positive relationship among the ischaemic time & perfusion time (bypass time) with the marker levels at all postoperative times for all markers.

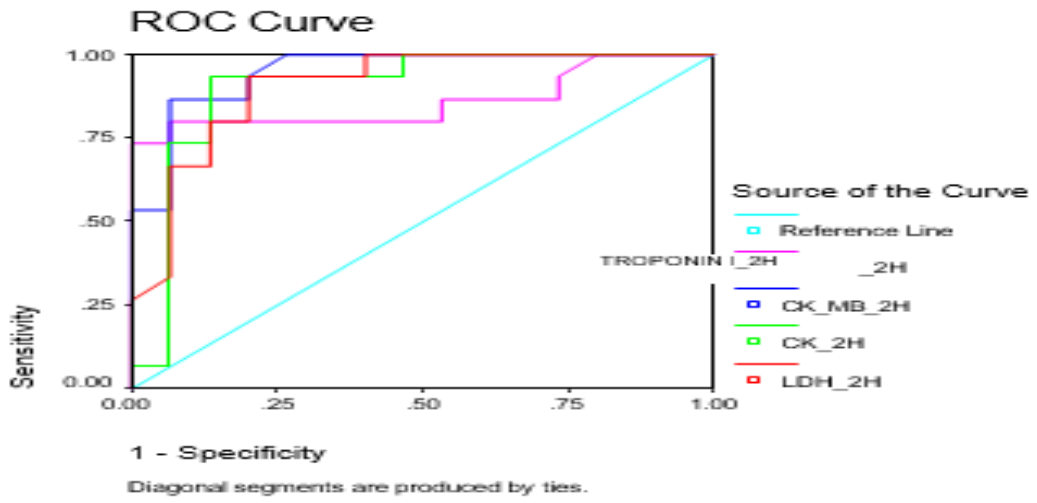

Figure (1): ROC curve comparing all markers at 2 hrs. after clamp release for group 2.

Area under the curve (AUC):
CK-MB = 0.949, LDH = 0.904, total CK =0.898, Troponin I = 0.860

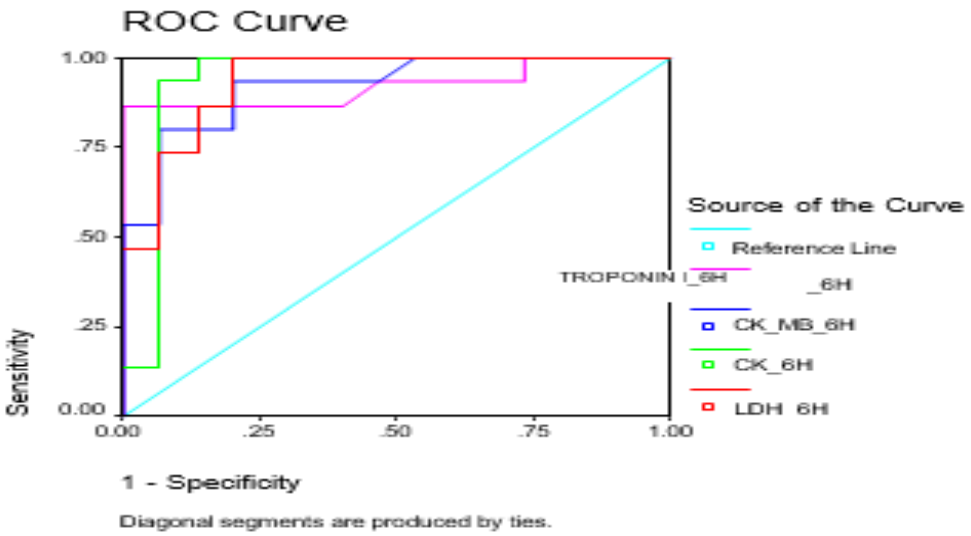

Figure (2): ROC curve comparing all markers at 6 hrs. after clamp release for group 2.

Area under the curve (AUC):
Total CK =0.938, LDH = 0.938, Troponin I = 0.922, CK-MB = 0.922

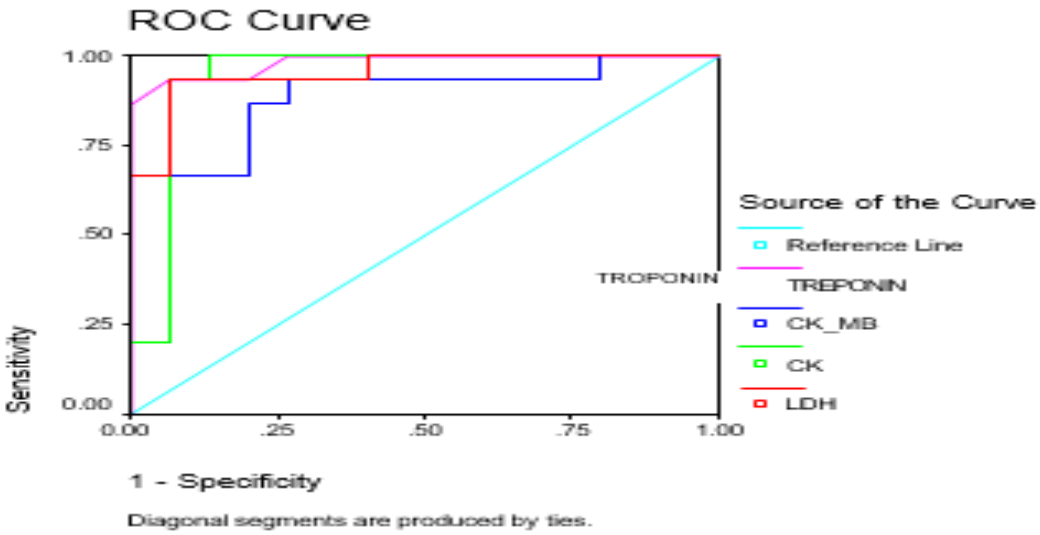

Figure (3): ROC curve comparing all markers at 20 hrs. after clamp release for group 2.

Area under the curve (AUC):
Troponin I = 0.982, LDH = 0.956, total CK = 0.942, CK-MB = 0.0.858

Discussion

One of the most unpredictable aspects of cardiac surgeries is the possibility of reperfusion damage. Reperfusion, which results in the end of ischaemia and is therefore necessary for the cell's survival and restoration of normal function, paradoxically causes damage to the cell. The term "ischaemia reperfusion injury" is used to describe both of

these injuries. 14

Cardiomyocyte cell biology has made amazing strides in recent years. Yet, keeping track of everything that goes on in these cells, particularly in terms of their function and malfunction, remains a challenge. 15

Four markers were chosen representing traditional (LDH), current (CK, CK-MB) and more recent (Troponin I) evolution in the study of cardiac biomarkers. The groups of patients were those subjected to VSD repair (group 1, age: 1-16 years, with postoperative ishchaemic changes) and valve replacement (group 2, age: 20-63 years, without postoperative ischaemic changes).

So this study was planned to monitor the level of some cardiac markers before (baseline) and 2 hrs,6 hrs, and 20 hrs after a period of myocardial ischaemia (cross-clamping) followed by reperfusion to assess the cardiac function in patients subjected to open heart surgery, along with monitoring through ECG and ECHO findings.

Non-significant differences are observed on comparison of the two groups. However significant increments are observed at different time intervals following aortic clamp release. That's because the reperfusion syndrome could be caused by cellular mechanisms such low levels of high-energy phosphates, an excess of oxygen-derived free radicals, membrane and mitochondrial damage, calcium accumulation in the heart, and acidosis. 16-18

The baseline value of total CK is not significantly different between the 2 groups but higher than the reference value. This was also in agreement with **Zhi.**[20] who reported a preoperative high total CK level in patients with VSD and with **Califf and Ohman**[19] **; Adams.** [21] who found that the younger the age, the higher the value of total CK.

Total CK activities are significantly higher in group 1 than group 2 at 2 hrs and 6 hrs after clamp release (Table 9), a finding which agrees with **Zhi.**[20] and could be due to the high vulnerability of the pediatric heart to the effect of ischaemia and reperfusion during repair of congenital heart disease as concluded by **Taggart.**[22] and **Hasegawa.**[23]

Due to the cellular mechanisms underlying the reperfusion syndrome, significant increments are observed at different time intervals following aortic clamp release (Table 11).[16-18]

The baseline value of Troponin I is insignificantly higher in group 1 than group 2 but within the reference value (Table 12).

Troponin I was found to be released in great amounts in V.S.D patients as was previously documented by **Dent CL**[24]

Troponin I levels are significantly higher in group 1 than in group 2 at 2 hrs and 6 hrs after clamp release (Table 12), a finding which agrees with **Jacquet.**[25] and could be attributed to the high vulnerability of the pediatric heart noted by **Taggart.**[22] and **Hasegawa.**[23]

Postoperative ischaemic ECG changes are reported in 100% of partients in group 1 and in 13.33 % in group 2 (Table 15). Statistical risk estimate (Table 16) shows that the VSD group are 7.5 times more vulnerable to ischaemic changes than the valve replacement group. This shows a high significance between both groups. This coincides with the findings of **Taggart**[22] and According to research conducted by **Hasegawa.23**, the effects of ischaemia and reperfusion on a child's heart are more severe than those experienced by an adult heart.

Correlation studies (Table 17) relating markers' levels to both ischaemic time and

the perfusion time (bypass time), show a positive correlation at all time intervals .Coincident with these findings, Extracorporeal circulation and aortic cross-clamping time are correlated with the severity of blood enzyme abnormalities during cardiopulmonary bypass, as reported by **Fransen.26 and Jaffe.27.**

Conclusions

The use of laboratory panels for assessment of an organ function is advocated by many clinicians. However, the sensitivity and specificity for each individual marker should be taken into consideration, in addition to cost-benefit relationship.

Laboratory markers of cardiac damage are now widely used, especially when false negative ECG changes are taken into consideration.

In place of CK and CK-MB, the recently released cardiac Troponin I has proven to be a sensitive and specific marker.

In comparison to an adult heart, a child's heart is more susceptible to damage from both ischaemia and reperfusion.

References

1. Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA. Ischemia/reperfusion injury. The Journal of surgical research. 2002;105(2):248-58.
2. Carden DL, Granger DN. Pathophysiology of ischaemia–reperfusion injury. The Journal of pathology. 2000 Feb;190(3):255-66.
3. Ganote C, Armstrong S. Ischaemia and the myocyte cytoskeleton: review and speculation. Cardiovascular research. 1993 Aug 1;27(8):1387-403.
4. Slavíková H, Lojek A, Hamar J, Dušková M, Kubala L, Vondráček J. Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. Free Radical Biology and Medicine. 1998 Jul 1;25(1):9-18.
5. Fan C, Zwacka RM, Engelhardt JF. Therapeutic approaches for ischemia/reperfusion injury in the liver. Journal of molecular medicine. 1999 Aug;77:577-92.
6. Reiter RJ. Oxidative damage in the central nervous system: protection by melatonin. Progress in neurobiology. 1998 Oct 1;56(3):359-84.
7. Semenza GL. Cellular and molecular dissection of reperfusion injury: ROS within and without. Circulation research. 2000 Feb 4;86(2):117-8.
8. Kaminski KA, Bonda TA, Korecki J, Musial WJ. Oxidative stress and neutrophil activation—the two keystones of ischemia/reperfusion injury. International journal of cardiology. 2002 Nov 1;86(1):41-59.
9. Maessen JG. Demonstration of ischemia-reperfusion injury separate from postoperative infarction in coronary artery bypass graft patients. The Annals of thoracic surgery. 1998 Jan 1;65(1):48-53.
10. Carrier M, Pellerin M, Perrault LP, Solymoss BC, Pelletier LC. Troponin levels in patients with myocardial infarction after coronary artery bypass grafting. The Annals of thoracic surgery. 2000 Feb 1;69(2):435-40.
11. Willging S, Keller F, Steinbach G. Specificity of cardiac troponins I and T in renal disease.
12. Landesberg G, Shatz V, Akopnik I, Wolf YG, Mayer M, Berlatzky Y, Weissman C, Mosseri M. Association of cardiac troponin, CK-MB, and postoperative myocardial

ischemia with long-term survival after major vascular surgery. Journal of the American College of Cardiology. 2003 Nov 5;42(9):1547-54.
13. Marshall T, Williams J, Williams KM. Electrophoresis of serum isoenzymes and proteins following acute myocardial infarction. Journal of Chromatography B: Biomedical Sciences and Applications. 1991 Sep 13;569(1-2):323-45.
14. Jones SP, Hoffmeyer MR, Sharp BR, Ho YS, Lefer DJ. Role of intracellular antioxidant enzymes after in vivo myocardial ischemia and reperfusion. American Journal of Physiology-Heart and Circulatory Physiology. 2003 Jan 1;284(1):H277-82.
15. Santander SF, Márquez MF, Duque MP, Sánchez RO, De la Peña Almaguer E, Lidt GE. Daño miocárdico por reperfusión. Rev Esp Cardiol. 2004;57(Supl 1):9-21.
16. Kober G, Buck T, Sievert H, Vallbracht C. Myocardial protection during percutaneous transluminal coronary angioplasty: effects of trimetazidine. European heart journal. 1992 Aug 1;13(8):1109-15.
17. Detry JM, Sellier P, Pennaforte S, Cokkinos D, Dargie H, Mathes P. Trimetazidine: a new concept in the treatment of angina. Comparison with propranolol in patients with stable angina. Trimetazidine European Multicenter Study Group. British journal of clinical pharmacology. 1994 Mar;37(3):279-88.
18. Tünerir B, Çolak Ö, Alataş Ö, Beşoğul Y, Kural T, Aslan R. Measurement of troponin T to detect cardioprotective effect of trimetazidine during coronary artery bypass grafting. The Annals of thoracic surgery. 1999 Dec 1;68(6):2173-6.
19. Califfi RM, Ohman EM. The diagnosis of acute myocardial infarction. Chest. 1992 Apr 1;101(4):106S-15S.
20. Zhi. Protective effect of Salvia Miltorrhizae Composita on myocardium in patients undergoing open heart surgery.1998; 18(2): 68-70.
21. Adams 3rd JE, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s?. Circulation. 1993 Aug;88(2):750-63.
22. Taggart DP, Hadjinikolas L, Hooper J, Albert J, Kemp M, Hue D, Yacoub M, Lincoln JC. Effects of age and ischemic times on biochemical evidence of myocardial injury after pediatric cardiac operations. The Journal of Thoracic and Cardiovascular Surgery. 1997 Apr 1;113(4):728-35.
23. Hasegawa T, Yamaguchi M, Yoshimura N, Okita Y. The dependence of myocardial damage on age and ischemic time in pediatric cardiac surgery. The Journal of thoracic and cardiovascular surgery. 2005 Jan 1;129(1):192-8.
24. Dent CL, Wood MK, Huddleston CB, Mendeloff EN, Balzer DT, Landt Y, Canter CE. Patterns and potential value of cardiac troponin I elevations after pediatric cardiac operations. The Annals of thoracic surgery. 1998 May 1;65(5):1394-9.
25. Jacquet L, Noirhomme P, El Khoury G, Goenen M, Philippe M, Col J, Dion R. Cardiac troponin I as an early marker of myocardial damage after coronary bypass surgery. European journal of cardio-thoracic surgery. 1998 Apr 1;13(4):378-84.
26. Fransen EJ, Maessen JG, Hermens WT, Glatz JF, Buurman WA. Peri-operative myocardial tissue injury and the release of inflammatory mediators in coronary artery bypass graft patients. Cardiovascular research. 2000 Mar 1;45(4):853-9.
27. Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, Katus H. It's time for a change to a troponin standard. Circulation. 2000 Sep 12;102(11):1216-20.