Evaluation of 6 cardiac troponin assays in patients with acute coronary syndrome

Salam M. Saadeddin, PhD, MT(ASCP), Moh'd A. Habbab, MD, FACC, Hisham H. Siddieg, BSc, Mohammed N. Al Seeni, MSc, Ashraf B. Tahery, MD, Samia H. Sobki, MD, Rofaida M. Dafterdar, MD.

ABSTRACT

Objectives: An increasing body of evidence has demonstrated the value of strategies based on cardiac troponin (cTn) assays in the diagnosis, prognosis and monitoring of patients with acute coronary syndrome (ACS). We evaluated the performance and the practicability of 6 commercially available assays (5 cTnI and 1 cTnT) in patients with ACS.

Methods: This study was carried out between October 2001 and June 2002 at Armed Forces Hospital in collaboration with Prince Sultan Cardiac Center, Riyadh, Kingdom of Saudi Arabia. Blood samples from 96 patients, 40 with and 56 without clinical evidence of myocardial injury, were used for the evaluation. Cardiac TnI assays were performed using 5 different immunoanalyzers AxSYM (Abbott Laboratories), Stratus CS (Dade Behring), ACS:180 (Bayer), Centaur (Bayer) and Immulite (Diagnostic Products Corporation) while cTnT was measured on the Elecsys (Roche) immunoanalyzer. The sensitivity, specificity and positive and negative predictive values (PV) were calculated for all assays. General and special features related to installation, routine operation, quality control and other various special parameters of each immunoanalyzer were evaluated (using the score of 1-5 for each parameter) and compared with those of other analyzers.

Results: The highest reliability values were observed with Immulite, followed by AxSYM, then Stratus, then ACS:180, and Centaur, and lastly by Elecsys. The highest practicability values were observed with Elecsys, and Centaur, followed by AxSYM and the lowest values were observed with Stratus.

Conclusion: Considering the combined performance and practicability score, the difference between the 10% coefficient variation cut off value and the 99th percentile value, and the difference in the relative reactivities to the various cTnI forms, the most favorable values were observed with AxSYM, followed by Immulite and Elecsys, then Centaur and Stratus, and lastly by ACS:180.

Saudi Med J 2003; Vol. 24 (10): 1092-1097

The troponin complex is a major component of the structural proteins involved in striated cardiac muscle contraction.[1,2] It is a heterotrimer consisting of troponins I, T, and C, which are tightly bound to the contractile apparatus; hence, circulating concentrations are low.[3,4] Troponin T functions to bind the troponin complex to the tropomysin strand; troponin I functions to inhibit the activity of actomyosin-adenosine triphosphate; and troponin C serves to bind 4 calcium ions, thus regulating contraction.[5,6] The cardiac isoforms of troponin I and troponin T are structurally different from the corresponding skeletal isoforms,[7-9] and therefore, they have recently established themselves as biochemical markers of myocardial damage. Measurements of these proteins have excellent performance characteristics for diagnosis of myocardial infarction,[10-13] risk stratification of acute coronary syndrome patients,[14-16] guidance of therapeutic intervention and prediction of outcome.[17-19] Several methods for the measurement of TnI and one method for

From the Department of Pathology (Saadeddin, Siddieg, Al Seeni, Sobki, Dafterdar), Armed Forces Hospital, Department of Adult Cardiology (Habbab, Tahery), Prince Sultan Cardiac Center, Riyadh, *Kingdom of Saudi Arabia*.

Received 30th April 2003. Accepted for publication in final form 15th July 2003.

Address correspondence and reprint request to: Dr. Salam M. Saadeddin, (R715) Armed Forces Hospital, PO Box 7897, Riyadh 11159, *Kingdom of Saudi Arabia*. Tel. +966 (1) 4609316. Fax. +966 (1) 4606016. E-mail: mhabbabksa@sps.net.sa

the measurement of cTnT are available in the market today.[20] The currently available cTnI assays produce differing results, which are related to the different recognition patterns of these immunoassays to the various forms of cTnI present in circulation or in biochemical preparations. These forms include: cTnI isoforms (free cTnI, TnI-C and TnI-C-T complexes), oxidized and phosphorylated forms of all the isoforms, and proteolytically degraded (modified) forms of cTnI which also may exist as binary or ternary complexes.[20]

The aim of this study was to compare the clinical performance and the practicability of 6 commercially available cardiac troponin assays in patients with acute coronary syndromes.

Methods. This study was carried out between October 2001, and June 2002 at Armed Forces Hospital in collaboration with Prince Sultan Cardiac Center, Riyadh, Kingdom of Saudi Arabia.

Patient samples. Ninety-six blood samples from patients with clinically proven coronary artery disease, with or without clinical evidence of myocardial injury, were used for the evaluation. The blood sample was centrifuged at 1900 g for 10 minutes, and the serum was frozen at -40^{0}C until the time of analysis. Clinical evidence of myocardial injury was diagnosed or ruled out by history, physical examination, echocardiogram and electrocardiogram review.

Immunoanalyzers. Cardiac TnI assays were performed using 5 different immunoanalyzers AxSYM (Abbott Laboratories), Stratus CS (Dade Behring), ACS:180 (Bayer), Centaur (Bayer) and Immulite (Diagnostic Products Corporation) while cardiac TnT was measured on the Elecsys (Roche) immunoanalyzer.

Assays. Assay protocol and platforms suggested by the manufacturers were used for all assays. All assays use a method where the analyte is sandwiched between capture and labeled antibodies (sandwich technique) and thus, the generated signal is directly proportional to the concentration of cTnI in the sample. The manufacturer, format/label type of the assay, types of capture and label antibodies, sample size, range and cutoff value for each analyzer are shown in **Table 1**. The number of assays performed on each immunoanalyzer varied according to the availability of kits. All 96 blood samples were tested on the Elecsys and the AxSYM, 56 samples (22 with and 34 without myocardial injury) on the Dade Behring Stratus II and 40 samples (18 with and 22 without myocardial injury) on the ACS:180 , and the Immulite.

Performance evaluation. The sensitivity, specificity, positive, and negative predictive values (PV) were calculated for all assays. Coefficient of variation (CV) values for intraassay (within-run) and interassay (between-days) precision were calculated for 2-3 levels of controls (low, medium and high) of each cTnT and cTnI assays preformed on different systems. Results of each control level in a single run were used for intraassay precision and results of each control level in different runs were used for interassay precision. Ten tests were carried out for each control level in a single run and in different runs.

Practicability evaluation. Parameters related to installation (all the processes necessary to make the system operative), routine operation (all the steps that

Table 1 - Comparison of the immunoassays used in the study.

Analyzer	AxSYM	Stratus CS	Immunize	ACS:180	Centaur	Elecsys
Manufacturer	Abbott Diagnostics, Abbott Park, IL	Dade Behring, Miami, FL	Diagnostic Products Corporation, Los Angeles, CA	Bayer Corp. Diagnostics Division, Walpole, MA	Bayer Corp. Diagnostics Division, Walpole, MA	Roche Diagnostics, Indianapolis, IN
Format/label type	Sandwich/ microparticle enzyme-fluorescent	Sandwich/ fluorescent	Sandwich/ chemiluminescent	Sandwich/ chemiluminescent	Sandwich/ chemiluminescent	Sandwich/electro chemiluminescent
Capture antibody	Mouse monoclonal antibody	Mouse monclonal antibody	Murine antibody	Two different mouse monoclonal antibodies	Two different mouse monoclonal antibodies	Monoclonal antibody
Label antibody	Goat polyclonal antibody	Mouse monoclonal antibody	Goat polyclonal antibody	Goat polyclonal antibody	Goat polyclonal antibody	Mouse monoclonal antibody
Sample size µl	200	220	50	100	100	250
Range µg/L	0.3-50	0.3-50	0.5-100	0.10-50	0.10-50	0.010-25.00
Cutoff for AMI µg/L	2.0	0.6	1.0	1.0	1.0	0.1

AMI - acute myocardial infarction

Table 2 - Distribution of the results of the assays.

Analyzer	n	Group A	Group B	True (+)	False (-)	True (-)	False (+)
AxSYM, cTnI	96	40	56	39	1	56	0
Stratus CS, cTnI	56	22	34	22	0	33	1
Immulite Turbo, cTnI	40	18	22	18	0	22	0
ACS: 180, cTn1	40	18	22	15	3	22	0
Centaur, cTnI	40	18	22	15	3	22	0
Elecsys, cTnT	96	40	56	36	4	53	3

n - number, Group A - patients with clinical evidence of myocardial injury
Group B - patients without clinical evidence of myocardial injury

Table 3 - Calculated parameters for the immunoassays used in the study.

Analyzer	Patients n	Sensitivity %	Specificity %	(+) Predictive value%	(-) Predictive value %
AxSYM, cTnI	96	(98)	(100)	(100)	(98)
Stratus CS, cTnI	56	(100)	(97)	(96)	(100)
Immulite Turbo, cTnI	40	(100)	(100)	(100)	(100)
ACS: 180, cTn1	40	(83)	(100)	(96)	(88)
Centaur, cTnI	40	(83)	(100)	(96)	(88)
Elecsys, cTnT	96	(90)	(95)	(92)	(93)

give the results), quality control (the calibration curve and the quality control) and other special features (important for the system management) of each immunoanalyzer were evaluated using a score of 1-5 for each parameter, with 5 being the most practical.

Results. A total of 96 consecutive patients (61 males and 35 females) with clinically proven coronary artery disease were studied. Their ages ranged from 31 to 77 years, with a mean age ± SD of 55.4 ± 14.5. Forty patients had clinical evidence of myocardial injury; 13 with unstable angina 27 with myocardial infarction (Group A); and the other 56 had stable coronary artery disease and no clinical evidence of myocardial injury (Group B). Cardiac TnT was found to be significantly elevated in 36/40 in group A and 3/56 in group B with 3 falsely positive and 4 falsely negative results. On the other hand, cTnI using the AxSYM, Stratus, Immulite, ACS:180 and Centaur analyzers, was found to be significantly elevated in 39/40, 22/22, 18/18, 15/18, and 15/18 in group A and 0/56, 1/34, 0/22, 0/22 and 0/22 in group B with 1, 0, 0, 3 and 3 falsely positive and 0, 1, 0, 0 and 0 falsely negative results **(Table 2).** The calculated sensitivity, specificity, and positive and negative predictive values for all assays are shown in **Table 3**. Immulite had the highest reliability values followed by AxSYM then Stratus then ACS:180 and Centaur and lastly by Elecsys. The calculated coefficient of variation (CV) values for intraassay (within-run) and interassay (between days) precision for all assays were <10 as shown in **Table 4**. Total, percentage and mean scores of different parameters used for the practicability evaluation are shown in **Table 5**. The highest practicability values were observed with Elecsys and Centaur followed by AxSYM and the lowest values were observed with Stratus.

Discussion. These results clearly demonstrate that cardiac troponins are highly sensitive and specific markers of cardiac injury. All cTnI assays showed higher specificity and positive predictive value for coronary artery disease than the cTnT assay. This difference in clinical specificity of both troponins supports previous reports.[21-24] Cardiac TnI occurs only in

Table 4 - Calculated coefficient of variation (CV) values.

Analyzer	Within-run CV's			Between-days CV's		
	Low %	Medium %	High %	Low %	Medium %	High %
AxSYM, cTnI	(8.2)	(7.6)	(6.8)	(8.6)	(6.3)	(5.8)
Stratus CS, cTnI	(1.2)	(2.9)	(1.9)	(6.5)	(3.7)	(2.2)
Immulite Turbo, cTnI	(6.8)	-	(3.5)	(8.7)	-	(7)
ACS: 180, cTn1	(7.6)	(4.2)	(6.8)	(7.1)	(6.9)	(7.1)
Centaur, cTnI	(1.2)	-	(3.6)	(7)	-	(2.6)
Elecsys, cTnT	(9.3)	(6.6)	(5.6)	(8)	(7.5)	(2.9)

Table 5 - Evaluation of the installation process.

Parameter	AxSYM	Stratus CS	Immulite	Centaur	ACS:180	Elecsys
Evaluation of installation process						
Space required	1	4	1	1	1	1
Software	4	2	3	4	4	4
Accessibility to switch reagent	5	1	5	5	5	3
Output of heat	1	3	2	1	2	1
Output of noise	1	3	1	1	1	2
Amount of liquid waste	3	5	4	2	2	4
Amount of solid waste	1	3	2	2	2	2
Total	**16**	**21**	**18**	**16**	**17**	**17**
Mean	2.3	3.0	2.6	2.3	2.4	2.4
Evaluation of routine operation						
Daily start up	4	4	3	4	1	5
Work list entered via keyboard	5	1	3	5	5	5
Reagents in chilled chamber	2	1	4	5	2	5
number of tests/kits	5	1	3	5	3	5
Sample preparation	4	5	4	4	4	4
Tests/hour	2	1	3	3	3	4
Random access	5	1	4	5	3	5
Working range	4	3	4	4	4	4
Total	**31**	**17**	**28**	**35**	**25**	**37**
Mean	3.9	2.1	3.5	4.4	3.1	4.6
Evaluation of quality control						
Stored master calibration curve	5	5	5	5	5	5
Recalibration procedure every 1-4 weeks	5	4	4	5	4	5
Quality control	4	5	3	4	3	4
Total	**14**	**14**	**12**	**14**	**12**	**14**
Mean	4.7	4.7	4.0	4.7	(4.0)	4.7
Evaluation of various parameters						
Software and hardware peculiarity	5	1	4	5	5	5
Connection to host computer	5	5	5	5	5	5
Troubleshooting	5	3	5	5	5	4
Technical assistance	5	5	5	5	5	5
Total	**20**	**14**	**19**	**20**	**20**	**19**
Mean	5	3.5	4.8	5.0	5.0	4.8
Total Score (Out of 110)	**81**	**66**	**77**	**85**	**74**	**86**
Total (%)	**(74)**	**(60)**	**(70)**	**(77)**	**(67)**	**(78)**
Mean Score (All parameters)	3.7	3.0	3.5	3.9	3.4	3.9
Mean Score (Mean values	3.98	3.33	3.73	4.1	3.63	4.13

Table 6 - Assay information.

Assay	LLD (μg/L)	99th% (μg/L)	10% CV con. (μg/L)	99th%/10% CV con.	AMI ROC (μg/L)
AxSYM	0.14	0.5	0.8	(63)	2
Stratus CS	0.03	0.06	0.13	(46)	0.6
Immulite Turbo	0.5	<0.5	0.6	-	1
ACS: 180	0.03	0.1	0.35	(29)	1
Centaur	0.02	0.1	0.35	(29)	1
Elecsys	0.01	0.01	0.035	(29)	0.1

LLD - lower limit of detection, 99th % - 99th percentile of the reference range, 10% CV conc. - concentration with ≤10% precision, AMI ROC - Acute myocardial infarction cutoff value determined by ROC analysis

myocardial cells.[21] Neither the protein nor its messenger ribonucleic acid have ever been detected outside of myocardial tissue at any point in ontogeny or in any pathologic state.[21] In multiple clinical evaluations, elevation of cTnI has been found only in patients with cardiac injury.[21,25-27] Although cTnT does demonstrate a relatively higher cardiac specificity, elevation of this protein in absence of cardiac injury have been described.[27] Multiple studies have found elevated levels of cTnT in the absence of discernible cardiac injury, most frequently in individuals with renal failure.[22,23] In our study the 3 patients with the false positive cTnT values had renal diseases with high creatinine levels. The 4 false negative values of cTnT occurred inpatients with unstable angina. These patients had small elevations in cTnI indicating the presence of minor myocardial injury in these patients. Such finding is in agreement with our previous reports of minor myocardial injury post cardiac intervention in patients with stable angina.[28,29] These findings suggest that cTnI is more specific than cTnT in detecting minor myocardial injury. The highest reliability values were observed with Immulite, followed by AxSYM, then Stratus, then ACS:180, and Centaur, and lastly by Elecsys,. The highest practicability values were observed with Elecsys, and Centaur, followed by AxSYM, and the lowest values were observed with Stratus.

The recently published new definition of acute myocardial infarction[30] by the joint committee of the European Society of Cardiology and the American College of Cardiology (ESC/ACC) recommends that the cardiac troponin cutoff values for diagnosis of acute myocardial infarction are the 99th percentile of the reference population at a level measured with analytic imprecision (CV<10%). Unfortunately, there are no commercially available troponin assays that meet these criteria. For the currently available assays, the ones that have the smallest difference between the 10% CV cutoff value and the 99th percentile value is expected to have the most reliable results. In addition, recent studies showed that increases of cardiac troponin values lower than the receiver operating curve (ROC) determined cutoff values (myocardial infarction cutoff values) but above the 10% CV cutoff value are associated with substantial increases in postevent morbidity and mortality.[31,32] Therefore, it is recommended by the ESC/ACC that a rise of cardiac troponin values above the value defined by the 10% CV be considered indicative of cardiac injury. Such an approach will provide highly sensitive detection of cardiac injury. **Table 6** shows the lower limit of detection, 99th percentile of the reference range, the 10% CV concentration (with ≤10% precision) and the acute myocardial infarction cut off value determined by ROC analysis for the studied assays.[33] From this table, Immulite, cannot give exact values below 0.5mg/L, which is the lower limit of detection, and the smallest difference between the 10% CV cutoff value and the 99th percentile value is that of the AxSYM, followed by the Stratus. Cardiac troponin I assays exhibit a differential response to reduced versus oxidized forms, therefore, they may exhibit changing results during the first few hours after blood collection, as all forms of cTnI can slowly oxidize to form disulfide linkages.[20] In addition, the oxidation of cTnI in circulating blood will yield a differential response among assays that do not recognize the oxidized and reduced forms equally.[20] Cardiac troponin I assays demonstrated different results when samples are not assayed immediately or after several hours of room temperature storage.[20] Since assays have different relative reactivities to the various cTnI forms, it is expected that the assay with the smallest difference in the relative reactivities will have the most reliable result. The difference in the relative reactivities to the various cTnI forms was studied by Wu et al[20] and was reported to be 2.5, 3, 4, 4.5 and 4.5 folds for AxSYM, Stratus, Immulite, ACS:180 and Centaur.

Considering the combined performance and practicability score, the difference between the 10% CV

cut off value and the 99th percentile value, and the difference in the relative reactivities to the various cTnI forms, the most favorable values were observed with AxSYM, followed by Immulite, and Elecsys, then Centaur, and Stratus, and lastly by ACS:180.

References

1. Zot AS, Potter JD. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. ***Annu Rev Biophys Biophys Chem*** 1987; 16: 535-539.
2. Leavis PC, Gergely J. Thin filament proteins and thin filament-linked regulation of vertebrate muscle contraction. ***CRC Crit Rev Biochem*** 1984; 16: 235-305.
3. Adams JE, Schechtman KB, Landt Y, Ladenson JH, Jaffe AS. Comparable detection of acute myocardial infarction by creatine kinase MB isoenzyme and cardiac troponin I. ***Clin Chem*** 1994; 40: 1291-1295.
4. Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich K W, Vinar G et al. The diagnostic efficiency of troponin T measurements in acute myocardial infarction. ***Circulation*** 1991; 83: 902-912.
5. Potter JB. Preparation of troponin and its subunits. ***Methods Enzymol*** 1982; 85: 241-263.
6. Staprans J, Takahashi H, Russell MP, Watanabe S. Skeletal and cardiac troponins and their components. ***J Biochem (Tokyo)*** 1973; 72: 723-735.
7. Wade R, Kedes L. Developmental regulation of contractile protein genes. ***Annu Rev Physiol*** 1989; 51: 179-188.
8. Wilkinson JM, Grand RJA. Comparison of amino acid sequence of troponin I from different striated muscles. ***Nature*** 1978; 271: 31-35.
9. Gusev NB, Barskaya NV, Verin AD, Duzhenkova IV, Khuchua ZA, Zheltova AO. Some properties of cardiac troponin T structure. ***Biochem J*** 1983; 213: 123-129.
10. Mair J, Morandell D, Genser N, Lechleitner P, Dienstl F, Puschendorf B. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. ***Clin Chem*** 1995; 41: 1266-1272.
11. Storrow AB, Gibler WB. Chest pain centers: diagnosis of acute coronary syndromes. ***Ann Emerg Med*** 2000; 35: 449-461.
12. Keffer JH. Myocardial markers of injury evolution and insights. ***Am J Clin Pathol*** 1996; 105: 305-320.
13. Christenson RH, Azzazy HME. Biochemical markers of the acute coronary syndromes. ***Clin Chem*** 1998; 44: 1855-1864.
14. Newby LK, Christenson RH, Ohman EM, Armstrong PW, Thompson TD, Lee KL et al. Value of serial troponin T measures for early and late risk stratification in patients with acute coronary syndromes. ***Circulation*** 1998; 998: 1853-1859.
15. Olatidoye AB, Wu AHB, Feng YJ, Waters D. Prognostic role of troponin T versus troponin I in unstable angina pectoris for cardiac events with meta-analysis comparing published studies. ***Am Heart J*** 1998; 81: 1405-1410.
16. Saadeddin SM, Habbab MA, Ferns GA. Cardiac markers for assessing the acute coronary syndromes. A focus on cardiac troponins. ***Saudi Med J*** 2000; 21: 228-237.
17. Hamm CW, Heechen C, Goldmann B, Vahanian A, Adgey J, Miguel CM et al. Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. ***N Engl J Med*** 1999; 340: 1634-1639.
18. Heeschen C, Hamm CW, Goldmann B, Deu A, Langenbrink L, White HD et al. Troponin concentration for stratification of patients with acute coronary syndromes in relation to therapeutic efficacy of tirofiban. ***Lancet*** 1999; 354: 1757-1762.
19. Lindahl B, Venge P. Troponin T identifies patients with unstable coronary artery disease who benefit from long-term antithrombotic protection. ***J Am Coll Cardiol*** 1997; 29: 43-48.
20. Wu AHB, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. ***Clin Chem*** 1998; 44: 1198-1208.
21. Bodor GS, Porterfield D, Voss EM, Smith S, Apple FS. Cardiac troponin I is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue. ***Clin Chem*** 1995: 41: 1710-1715.
22. Braun SL, Baum H, Neumeier D, Vogt W. Troponin T and troponin I after coronary artery bypass grafting: Discordant results in patients with renal failure. ***Clin Chem*** 1996; 42: 781.
23. Heller C, Zehelein J, Remppis A, Kubler W, Katus HA. Cardiac troponin T in patients with renal failure. ***J Am Coll Cardiol*** 1997; 29: 234A.
24. Keffer JH. Myocardial markers of injury, evolution and insights. ***Am J Clin Pathol*** 1996; 105: 305-320.
25. Adams JE, Bodor GS, Davila-Roman VG, Delmez JA, Apple FS, Ladenson JH et al. Cardiac troponin I, a marker with high specificity for cardiac injury. ***Circulation*** 1993; 88: 101-106.
26. Benoist JF, Cosson C, Mimoz O, Edouard A. Serum cardiac troponin I, creatine kinase (CK), and CK-MB inearly post traumatic rhabdomyolysis. ***Clin Chem*** 1997; 43: 416-417.
27. Braun SL, Pongratz DE, Bialk P, Liem S, Schlotter B, Vogt W. Discrepant results for cardiac troponin T and troponin I in chronic myopathy, depending on instrument and assay generation. ***Clin Chem*** 1996; 42: 2039.
28. Saadeddin SM, Habbab MA, Sobki SH, Ferns GA. Detection of minor myocardial injury after successful percutaneous transluminal coronary angioplasty with or without stenting. ***Med Sci Monit*** 2000; 6: 708-712.
29. Saadeddin SM, Habbab MA, Sobki SH, Ferns GA. Minor myocardial injury after lective uncomplicated successful PTCA with or without stenting: Detection by cardiac troponins. ***Catheter Cardiovasc Interv*** 2001; 53: 188-192.
30. Alpert JS, Thygesen K, Antman E, 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.
31. Cannon CP, Weintraub WS, Demopoulos LA, Vicari R, Frey MJ, Lakkis N et al. Comparison of early invasive and conservative strategies in patients with unstable coronary syndromes treated with the glycoprotein IIb/IIIa inhibitor tirofiban. ***N Engl J Med*** 2001; 244: 1879-1887.
32. FRISC II investigators. Invasive compared with non-invasive treatment in unstable coronary artery disease: FRISC II prospective randomised multicentre study. Fragmin and Fast Revascularisation during InStability in Coronary artery disease Investigators. ***Lancet*** 1999; 345: 708-715.
33. Apple FS, Wu AHB, Jaffe AS. European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: how to use existing assays clinically and for clinical trials. ***Am Heart J*** 2002; 144: 981-986.