[Frontiers in Bioscience E4, 2322-2327, June 1, 2012]

High sensitive troponin T in individuals with chest pain of presumed ischemic origin

Giovanni Cuda[1], Margherita Lentini[1], Luigia Gallo[1], Fortunata G. Lucia[1], Lorenzina Giaquinto Carinci[1], Serafina Mancuso[1], Rosa A. Biondi[1], Raffaella Sinopoli[1], Rita Casadonte[1], Pietro H. Guzzi[2], Mario Cannataro[2], Annalisa Mongiardo[3], Claudio Iaconetti[3], Angela Bochicchio[3], Antonio Curcio[3], Daniele Torella[3], Pietroantonio Ricci[4], Ciro Indolfi[3], Francesco Costanzo[1]

[1]Laboratory of Clinical Biochemistry and Molecular Biology, Fondazione, T. Campanella, University of Magna Graecia, Catanzaro, Italy, [2]Laboratory of Bioinformatics, University of Magna Graecia, Catanzaro, Italy, [3]Division of Cardiology, University of Magna Graecia, Catanzaro, Italy, [4]Division of Forensic Medicine, University of Magna Graecia, Catanzaro, Italy

TABLE OF CONTENTS

1. ABSTRACT

This study was aimed at assessing the bias of high sensitive cardiac troponin T vs. the standard cardiac troponin T in a selected population with chest pain of presumed cardiac origin. Serum cTnT was determined in 132 patients and in 106 apparently healthy controls by both assays. The hs-cTnT outperformed the standard generation assay by: i) allowing a larger and earlier diagnosis of AMI (74.2% vs. 64.3% patients resulted positive at the final diagnosis of AMI when tested with the hs-cTnT or the std-cTnT assay, respectively); ii) showing a better time-dependent dynamics in patients with AMI due to a higher precision at low concentrations; iii) identifying, within the controls, 6 subjects in whom a further examination revealed the presence of chronic asymptomatic cardiac ischemia. The results underscore the excellent performance of the hs-cTnT assay in our population. The use of this test can thus be strongly recommended in subjects presenting to the emergency unit with chest pain of presumed ischemic origin in order to increase the probability of earlier diagnosis of AMI, especially in non-STEMI.

2. INTRODUCTION

Acute myocardial infarction (AMI) is a disease characterized by death of myocardial cells due to a prolonged ischemia that overwhelms the physiological mechanisms of cell repair. Ischemia may occur as a consequence of increased myocardial metabolic demand, decreased delivery of oxygen to the cardiac muscle, or both.According to the Joint ESC-ACCF-AHA-WHF Task Force for the Redefinition of myocardial infarction, AMI can be diagnosed when cardiac troponin (cTn) is present and detectable in the blood of an individual exhibiting signs and symptoms of myocardial infarction (1).Incidence of AMI is still very high: in Italy it is estimated that 130000 new cases of AMI occur each year; of those, about 33000 die and, in more than 18000 cases, the death occurs prior to reaching the hospital.

Rapid, reliable and sensitive diagnostic tools are therefore highly needed for management optimization of these critical patients. Electrocardiography is the most important and widely used approach to complement clinical

Table 1. Baseline characteristics of the patients

Characteristics	Patients (No. 132)
Demographic characteristics	
Age – yr	66,9±11,6
Male sex - no. (%)	91 (68,9)
Female sex - no. (%)	41 (31,1)
Mean body mass index	27,4±4,2
Medical history	
Previous myocardial infarction - no. (%)	30 (22,7)
Previous revascularization - no. (%)	34 (24,2)
Coronary artery disease - no. (%)	63 (47,7)
Previous or current hypertension - no. (%)	42 (31,8)
Hypercholesterolemia - no. (%)	51 (38,6)
Diabetes mellitus - no. (%)	17 (12,8)
Previous stroke - no. (%)	4 (3)
Smoking - no. (%)	48 (36,3)
Mean blood pressure - mmHg	
Systolic	139,4±14,8
Diastolic	85,2±7,9
Mean heart rate – beats/min	76±6
Electrocardiographic findings – no. (%)	
ST-segment elevation	37 (28)
ST-segment depression	24 (18,1)
Left bundle-branch block	7 (5,3)
T-wave inversion	18 (13.6)
No significant abnormalities	13 (9.8)

assessment in the diagnosis of AMI (2), but is often insufficient due to the fact that electrocardiographic patterns suggestive of myocardial ischemia/necrosis can be detected in several non-AMI conditions (3, 4). Detection of the highly specific cTn T or I proteins in the serum of individuals with a clinical suspect of myocardial infarction has been shown to improve significantly the diagnosis of cardiac injury (3, 5-7), displaying a higher sensitivity than other biomarkers, such as creatin kinase MB and myoglobin (8, 9). In healthy subjects, serum cTn level is estimated to be within the range of 0.0001-0.0002 µg/L, due to a physiological loss of cardiomyocytes ($45x10^6$/year in the left ventricle) (10, 11). Changes in the 20% range of concentration of serum cTn are generally considered significant to differentiate between acute and chronic cardiac accidents (12); however, several reports suggest that a higher increment may be required (13). An important limitation of standard cTn assays resides in their low sensitivity, leading to a delay in confirming the diagnosis of AMI, with many drawbacks in the clinical management of the affected patients.

Very recently, high sensitivity cTn diagnostic tests have been released by several companies, which allow to further increase the detection limit of this protein in the circulating blood at the 99th percentile of an apparently healthy reference population with <10% coefficient of variation (CV), therefore complying with the requirements of the ESC-ACCF-AHA-WHF Task Force and the Study Group on Biomarkers in Cardiology of the Working Group on Acute Cardiac Care of the European Society of Cardiology (1, 14).

In the present study, we show the results of a comparative analysis of the 4th generation cTnT assay (std-cTnT) with the high sensitivity cTnT (hs-cTnT) assay (Elecsys Troponin T hs, Roche Diagnostics), performed on 132 samples from patients referred to us from the Cardiology Unit, as well as from 106 randomly selected and apparently healthy individuals.

3. MATERIALS AND METHODS

3.1. Patients population

The study has been conducted in the Clinical Biochemistry Laboratory of the Magna Graecia University, School of Medicine, Catanzaro (Italy), from March 2009 through February 2010. The study has been cleared by the Institution Ethics Review Board for human studies and patients have signed an informed consent. The patients enrolled in the study ranged from 29 to 88 years of age, and were admitted to the Emergency Unit (EU) because of an episode of chest pain of presumed ischemic origin, lasting for at least 5 minutes, but less than 6 hours, within the previous 24 hours. Baseline characteristics are reported in Table 1. Patients were excluded from the study in the presence of a documented myocardial infarction within the previous three weeks, thrombolytic therapy or angioplasty within the previous 6 months. Unstable angina or non–Q-wave myocardial infarction were diagnosed on the basis of serial electrocardiograms and determinations of creatine kinase or CK-MB. Blood sample collection was performed at the time of admission and after 6, 9 and 12 hours.

3.2. Specimen collection and measurement of Cardiac Troponin T

Serum specimens were collected in standard tubes or tubes containing separating gel and immediately subjected to analysis accordingly with the manufacturer's specifications. For the purposes of this study, the analysis was performed on the Cobas e411/e 601 analyzers (Roche Diagnostics) under the following conditions: std-cTnT with a detection limit of 0.01 µg/L, a 99th-percentile cutoff point of less than 0.01 µg/L and a coefficient of variation of less than 10% at 0.035 µg/L; hs-cTnT with a detection limit of 0.002 µg/L, a 99th-percentile cutoff point of less than 0.014 µg/L and a coefficient of variation of less than 10% at 0.013 µg/L. As recommended by the Study Group on Biomarkers in Cardiology of the Working Group on Acute Cardiac Care of the European Society of Cardiology, the decision limit for cardiac injury was established as the concentration of cTnT (either std-cTnT or hs-cTnT) that corresponds to the 99th percentile limit of the reference distribution in a sex- and age-matched healthy reference population. Diagnosis of myocardial necrosis was made on the basis of a rising and falling cTnT pattern, with at least one value above the 99th percentile. All samples were measured as a single determination.

3.3. Statistical analysis

Data analysis was done by the use of the Pearson chi-square test and the Fisher exact test. Nonparametric analysis was performed for comparison of assays. The criterion for significance was P<0.05. Continuous variable are presented as means (±SD).

4. RESULTS

A total of 238 individuals were enrolled in the study: of these, 132 patients had been admitted to the EU, while the remaining 106 were apparently healthy individuals. The mean (±SD) age was 66,9±11,6 and 65,6±17,3 for patients and healthy controls, respectively;

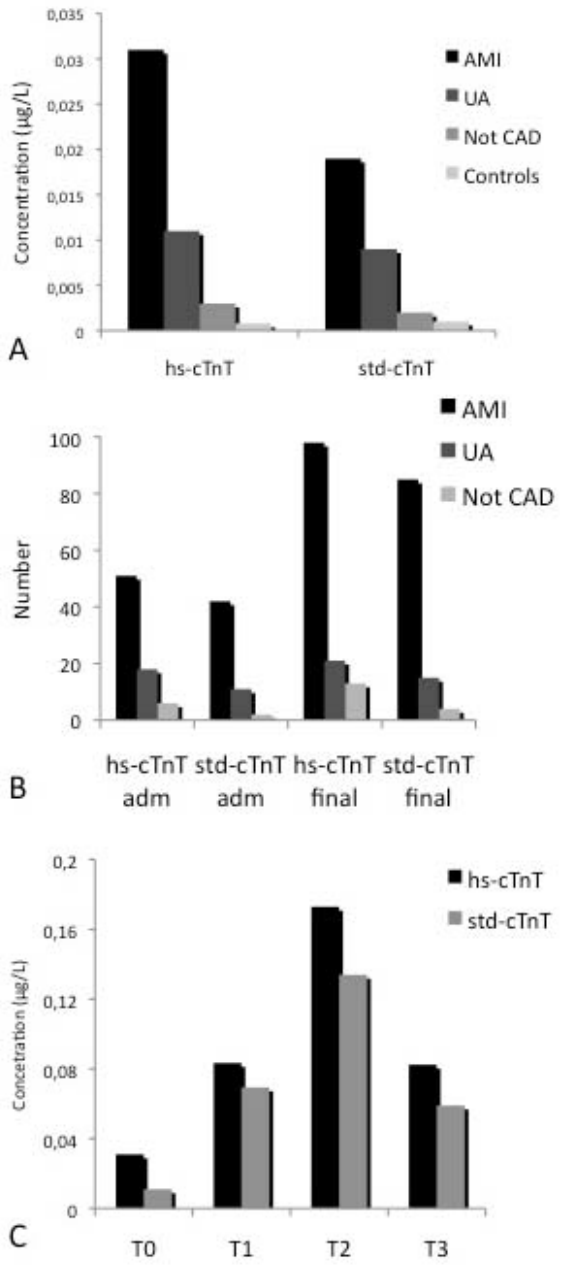

Figure 1. 1A: cTnT levels (mean+SD) measured by the hs-cTnT and the sdt-cTnT assays in patients with acute myocardial infarction (AMI), unstable angina (UA), non coronary artery diseases (Not CAD) and in apparently healthy subjects (Controls). 1B: Distribution of cTnT values in the patient population. 1C: Time dependent dynamics of cTnT levels (mean+SD) measured with the hs-cTnT and the std-cTnT in patients with AMI. T0: admission; T1: 6 h; T2: 9 h; T3: 12h

68,9% man and 31,1% women in the patient's cohort, 64,7% man and 35,3% women in the control group. The presence of AMI was confirmed in 98 patients (74.2%), in 21 a diagnosis of unstable angina was made (15.9%); the remaining 13 patients (9.8%) were classified as affected by non coronary artery disease (non-CAD). At presentation, both std-cTnT and hs-cTnT assays showed more elevated troponin levels in patients who underwent AMI compared to individuals with unstable angina or non-CAD (Figure 1A). As expected, the diagnostic accuracy of hs-cTnT was very high at presentation; the high sensitivity assay outperformed the standard one detecting the presence of cTnT in the blood of AMI patients earlier and longer than the std-cTnT (P<0.05) (Figure 1B and 1C). The adjudicated final diagnosis of AMI significantly increased from 64.3% to 74.2% in patients tested with the std-cTnT vs. the hs-cTnT assay, respectively (P<0.02).

hs-cTnT and std-cTnT levels measured in the 132 patients and 106 apparently healthy controls are reported in Table 2. The performance of hs-cTnT was significantly higher with respect to the std-cTnT assay: in particular, with the hs-cTnT assay, concentrations of cTnT were at or above the limit of detection (0.002 µg/L) in 292 out of 294 determinations (99.3%) and at or above the 99th percentile (0.014 µg/L) in 245 out of 294 determinations (83.3%) (P<0.05). On the other side, the std-cTnT assay gave the following results: 234 out of 294 determinations (79.6%) were at or above the limit of detection (0.01 µg/L), 221 out of 294 (75.1%) were at or above the 99th percentile (P<0.02). In the 106 apparently healthy subjects enrolled in the study as controls, hs-cTnT was at or above the detection limit in 20 cases (18.9%) and at or above the 99th percentile in 16 cases (9.4%), while the std-cTnT assay resulted at or above the detection limit in 4 cases (3.8%) and at or above the 99th percentile in 6 cases (5.7%) (Table 3). The distribution of cTnT levels, as measured by the high and the standard sensitivity assay in patients is shown in Figure 2A. A fair degree of concordance between the two assays emerged from the overall measuring range, but a significant difference was observed at the very low end, both in the patient (Figure 2B) and in the control population (Figure 2C) (P<0.05), suggesting that, below 0,01 µg/L, determinations obtained with std-cTnT and hs-cTnT cannot be directly compared.

5. DISCUSSION

A correct risk assessment of patients referred to the EU because of acute chest pain is a daily challenge, even for expert physicians; this is due to the wide variety of symptoms, especially in individuals with non-ST elevation acute coronary syndrome, and to the low specificity of instrumental diagnostic tools (i.e. electrocardiography). The availability of reliable, sensitive and specific markers of cardiac necrosis is therefore highly desirable. The development during the past 10-15 years of cTn assays has represented a major advancement in the diagnosis of AMI. The detection of cTnT and I subunits in the circulating blood has significantly increased the percentage of AMI cases correctly identified; moreover, many reports clearly demonstrate that elevation of cTn levels can be correlated to the extent of the necrotic area and that its detection may substantially improve the early diagnosis of AMI, especially in patients with a recent onset of chest pain (15). According to the recent literature, the definition of increased levels of cTn can be made only when they exceed

Table 2. hs-cTnT and std-cTnT values in patients and apparently healthy individuals

Assay	Patients (mean+SD)			Controls (mean+SD)		
	Total	Male	Female	Total	Male	Female
hs-cTnT (µg/L)	0,0760±0,0155	0,0885±0,030	0,0457±0,019	0,008±0,001	0,009±0,0002	0,007±0,0003
std-cTnT (µg/L)	0,0789±0,0126	0,0919±0,029	0,0476±,013	0,01±0,001	0,010±0,0002	0,010±0,0004

Table 3. Diagnostic performance of hs-cTnT vs. std-cTnT. LoD: limit of detection

Assay	Patients		Controls	
	= or > LoD (%)	= or > 99° percentile (%)	= or > LoD (%)	= or > 99° percentile (%)
hs-cTnT	99,3	83,3	18,9	9,4
std-cTnT	79,6	75,1	3,8	5,7

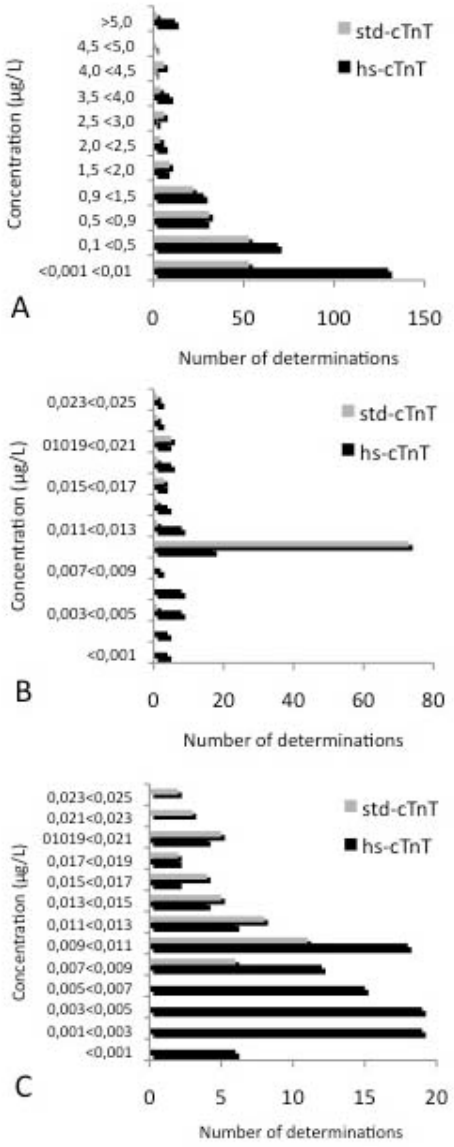

Figure 2. 2A: Distribution of cTnT levels over a wide range of concentration (<0.001>5.0 µg/L) measured by the hs-cTnT and the std-cTnT assays in the patient population. 2B: Distribution of cTnT within the concentration range <0.001<0.025 µg/L) measured by the hs-cTnT and the std-cTnT assays in the patient population. 2C: Distribution of cTnT within the concentration range <0.001<0.025 µg/L) measured by the hs-cTnT and the std-cTnT assays in the control population

the 99th percentile upper reference limit in a healthy reference population (CV<10%) (3, 16-18). To this end, more sensitive assays are needed. Very recently, several companies have released new, better performing cTn assays, which can be used for an earlier and more accurate identification of AMI patient, as well as for a better risk stratification.

In the present study we evaluated two different cTnT assays: the 4th generation cTnT assay (std-cTnT) and the high sensitivity cTnT (hs-cTnT) assay (both from Roche Diagnostics). The diagnostic performance of the hs-cTnT was significantly higher than that of the std-cTnT assay. Hs-cTnT was, in fact, able to detect the presence of circulating cTnT earlier and longer than the standard assay in AMI patients. Moreover, hs-cTnT outperformed the std-cTnT assay in the correct identification of AMI vs. non-AMI patients (i.e. individuals affected by unstable angina and/or non coronary artery disease). Remarkably, with the hs-cTnT assay, 292 out of 294 determinations (99.3%) were above the limit of detection (0.002 µg/L) in the patient population, while only 234 out of 294 (79.6%) overcame the limit of detection (0,01 µg/L) in the same population when the standard cTnT assay was used. We further compared the analytical performance of the two assays by studying the distribution of cTnT levels and found that, even if hs-cTnT and std-cTnT show a reasonable concordance over a wide range of concentrations, the 4th generation assay cannot provide reliable data below the 0.01 µg/L limit, leading to either an underestimation or a late diagnosis of AMI patients. Interestingly, the high sensitivity assay, applied to an apparently healthy cohort (106 individuals) in which clinical conditions potentially associated to non-ischemic myocardial damage, as well as non-cardiac cTnT-associated diseases were excluded, was able to identify six subjects in whom a further and accurate examination revealed the presence of chronic asymptomatic cardiac ischemia. This finding, which is still under investigation, appears of particular interest and highlights to the usefulness of the hs-cTnT test in a specific subset of individuals with silent cardiac ischemia.

All together, our results underscore the excellent performance of the hs-cTnT assay in the studied population compared to the 4th generation assay. Even small elevations of cTnT, undetectable with the standard assay, have been reported to be

associated to a worse outcome during follow up (higher incidence of cardiovascular death and heart failure), even in subjects with stable coronary artery disease (19). Thus, the availability of sensitive, specific and accurate tools is highly desirable. It must be considered that this is an observational, retrospective analysis of a relatively small cohort of patients admitted to the EU and therefore, as pointed out in similar studies, definitive conclusions cannot be drawn. However, we feel that our findings are sufficient to strongly encourage the use of high sensitive cardiac troponin assays in subjects presenting to the emergency department in order to increase the likelihood of improving early diagnosis of AMI, especially in non-ST elevation acute coronary syndrome.

6. REFERENCES

1. J S Alpert, K Thygesen, E Antman, JP Bassand: 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* 36, 959-69 (2000)

2. E. M. Antman, M. Hand, P. W. Armstrong, E. R. Bates, LA Green, LK Halasyamani, JS Hochman, HM Krumholz, GA Lamas, CJ Mullany, DL Pearle, MA Sloan, SC jr Smith; 2004 Writing Committee Members, DT Anbe, FG Kushner, JP Ornato, AK Jacobs, CD Adams, JL Anderson, CE Buller, MA Creager, SM Ettinger, JL Halperin, SA Hunt, BW Lytle, R Nishimura, RL Page, B Riegel, LG Tarkington, CW Yancy: 2007 Focused Update of the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: developed in collaboration With the Canadian Cardiovascular Society endorsed by the American Academy of Family Physicians: 2007 Writing Group to Review New Evidence and Update the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction, Writing on Behalf of the 2004 Writing Committee. *Circulation* 117, 296-329 (2008)

3. K Thygesen, JS Alpert, HD White: Universal definition of myocardial infarction. *Eur Heart J* 28, 2525-38 (2007)

4. K Wang, RW Asinger, HJ Marriott: ST-segment elevation in conditions other than acute myocardial infarction. *N Engl J Med* 349, 2128-35 (2003)

5. JP Bassand, CW Hamm, D Ardissino, E Boersma, A Budaj, F Fernández-Avilés, KA Fox, D Hasdai, EM Ohman, L Wallentin, W Wijns: Task Force for Diagnosis and Treatment of Non-ST-Segment Elevation Acute Coronary Syndromes of European Society of Cardiology: Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur Heart J* 28, 1598-660 (2007)

6. PA Kavsak, AM Newman, V Lustig, AR MacRae, GE Palomaki, DT Ko, JV Tu, AS Jaffe: Long-term health outcomes associated with detectable troponin I concentrations. *Clin Chem* 53, 220-227 (2007)

7. KM Eggers, B Lagerqvist, P Venge, L Wallentin, B Lindahl: Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. *Circulation* 116, 1907-1914 (2007)

8. W Hochholzer, HJ Buettner, D Trenk, K Laule, M Christ, FJ Neumann, C Mueller: New definition of myocardial infarction: impact on long-term mortality. *Am J Med* 121, 399-405 (2008)

9. KM Eggers, J Oldgren, A Nordenskjöld, B Lindahl: Diagnostic value of serial measurement of cardiac markers in patients with chest pain: limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. *Am Heart J* 148, 574-581 (2004)

10. VC Vasile, AK Saenger, JM Kroning, AS Jaffe: Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. *Clin Chem* 56, 1086-90 (2010)

11. LM Buja, D Vela: Cardiomyocyte death and renewal in the normal and diseased heart. *Cardiovasc Pathol* 17, 349-74 (2008)

12. K Thygesen, JS Alpert, HD White: Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction: Universal definition of myocardial infarction. *J Am Coll Cardiol* 50, 2173-2195 (2007)

13. AH Wu, QA Lu, J Todd, J Moecks, F Wians: Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. *Clin Chem* 55, 52-58 (2009)

14. K Thygesen, J Mair, H Katus, M Plebani, P Venge, P Collinson, B Lindahl, E Giannitsis, Y Hasin, M Galvani, M Tubaro, JS Alpert, LM Biasucci, W Koenig, C Mueller, K Huber, C Hamm, AS Jaffe: Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care: Recommendations for the use of cardiac troponin measurement in acute cardiac care. *Eur Heart J* 31(18), 2197-2204 (2010)

15. T Reichlin, W Hochholzer, S Bassetti, S Steuer, C Stelzig, S Hartwiger, S Biedert, N Schaub, C Buerge, M Potocki, M Noveanu, T Breidthardt, R Twerenbold, K Winkler, R Bingisser, C Mueller: Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med* 361, 858-867 (2009)

16. AK Saenger, AS Jaffe: Requiem for a heavyweight: the demise of creatine kinase-MB. *Circulation* 118, 2200-2206 (2008)

17. FS Apple, AH Wu, AS Jaffe, M Panteghini, RH Christenson, CP Cannon, G Francis, RL Jesse, DA Morrow, LK Newby, AB Storrow, WH Tang, F Pagani, J Tate, J Ordonez-Llanos, J Mair: National Academy of Clinical Biochemistry; IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of markers of cardiac damage laboratory medicine practice guidelines: Analytical issues for biochemical markers of acute coronary syndrome. *Circulation* 115, e352-e355 (2007)

18. E Giannitsis, H Katus: Current recommendations for interpretation of the highly sensitive troponin T assay for diagnostic, therapeutic and prognostic purposes in patients with Non-ST-segment-elevation acute coronary syndrome. *European Cardiology* 5, 44-47 (2009)

19. T Omland, JA. de Lemos, MS Sabatine, CA Christophi, MM Rice, KA Jablonski, S Tjora, MJ Domanski, BJ Gersh, JL Rouleau, M. A. Pfeffer, E. Braunwald: Prevention of Events with Angiotensin Converting Enzyme Inhibition (PEACE) Trial Investigators: A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 361, 2538-2547 (2009)

Abbreviations: cTn, cardiac troponin; hs-cTnT, high sensitive cardiac troponin T; std-cTnT, standard cardiac troponin T; AMI, acute myocardial infarction; UA, unstable angina; Not-CAD, not coronary artery disease; SD, standard deviation

Key Words: Cardiac troponin T, Acute myocardial infarction, Coronary artery disease, Early diagnosis, Biomarkers

Send correspondence to: Giovanni Cuda, Laboratory of Clinical Biochemistry and Molecular Biology, Fondazione,T. Campanella, University of Magna Graecia, Germaneto University Campus, 88100 Catanzaro, Italy, Tel: 39 0961 3694225, Fax: 39 0961 3694073, E-mail: cuda@unicz.it