

**DIPYRIDAMOLE STRESS ECHOCARDIOGRAPHY DOES NOT TRIGGER
RELEASE OF HIGHLY-SENSITIVE TROPONIN I AND T**DIPYRIDAMOLSKA STRES-EHOKARDIOGRAFIJA NE POKREĆE OSLOBAĐANJE
VISOKOOSETLJIVOG TROPONINA I I TGianfranco Cervellin¹, Fiorenza Robuschi¹, Francesco Scioscioli¹, Livia Ruffini²,
Mariella Dipalo³, Gian Luca Salvagno⁴, Giuseppe Lippi³¹Emergency Department, Academic Hospital of Parma, Parma, Italy²Department of Radiology, Academic Hospital of Parma, Parma, Italy³Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy⁴Laboratory of Clinical Chemistry and Hematology, University of Verona, Verona, Italy**Summary**

Background: The patients with episodes of chest pain and no electrocardiographic or biomarker abnormalities are currently monitored and subjected to non-invasive testing. Stress echocardiography is one of the most often used provocative tests, being the most cost- and risk-effective imaging technique. Some concerns about this technique have been raised regarding potential drug-induced myocardial injury. Our study hence aimed to establish whether or not dipyridamole stress echocardiography elicits release of troponin I (TnI) and T (TnT), as reliable biomarkers of myocardial injury.

Methods: Thirty-two patients, after exclusion of ongoing acute coronary syndrome (ACS) during evaluation in the emergency department (ED), were studied with echocardiography both at the baseline and after pharmacological stress with dipyridamole.

Results: All subjects had biomarkers assessment immediately before the stress-test (T1), 1 h from conclusion of the test (T2), and 6 h afterwards (T3). Cardiospecific troponins were assessed with one contemporary-sensitive (TnI) and two highly-sensitive (HS) methods (HS-TnI and HS-TnT). The concentration of TnI, HS-TnI and HS-TnT did not differ throughout the three time points. At no time point the concentration of either HS-TnI or HS-TnT was significantly different among patients with negative or positive stress test.

Kratak sadržaj

Uvod: Pacijenti sa epizodama bola u grudima, kod kojih elektrokardiografijom i pomoću biomarkera nisu otkriveni poremećaji, danas se prate i podvrgavaju neinvazivnom testiranju. Jedan od najčešće korišćenih provokativnih testova je stres-ehokardiografija, kao finansijski najisplativija i najmanje rizična *imaging* tehnika. Izvesne nedoumice u vezi sa ovom tehnikom odnose se na potencijalna farmakološki izazvana oštećenja miokarda. Cilj naše studije bio je da se ustanovi da li dipiridamolska stres-ehokardiografija izaziva ili ne izaziva oslobađanje troponina I (TnI) i T (TnT) kao pouzdanih biomarkera oštećenja miokardiocita.

Metode: Pošto su tokom evaluacije u hitnoj službi (HS) isključeni pacijenti sa akutnim koronarnim sindromom (AKS), pre i posle podvrgavanja farmakološkom stresu dipiridamolom ehokardiografski je testirano trideset dvoje pacijenata.

Rezultati: Biomarkeri su određeni kod svih ispitanika neposredno pre stres-testa (T1), 1 h po završetku testa (T2) i posle 6 h (T3). Kardiospecifični troponini su određeni pomoću jedne savremeno-osetljive (TnI) i dve visokoosetljive (VO) metode (HS-TnI i HS-TnT). Koncentracije TnI, HS-TnI i HS-TnT nisu se razlikovale u vreme merenja. Ni u jednom trenutku merenja koncentracije HS-TnI i HS-TnT se nisu razlikovale između pacijenata sa negativnim ili pozitivnim rezultatom stres-testa.

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Conclusions: The data shows that dipyridamole stress testing does not trigger release of troponin in patients with and without inducible reversible ischemia.

Keywords: troponin, stress test, echocardiography, stress echo, dipyridamole

Introduction

Coronary artery disease (CAD) is the most frequent cause of mortality in developed countries, accounting for nearly 12.8% of all deaths. In Europe, one in six males and one in seven females die from acute myocardial infarction (AMI) (1). For emergency physicians, chest pain is a high-frequency, high-risk, chief complaint, which represents also a leading cause for Emergency Department (ED) visits worldwide. In agreement with the reasonable assumption that *»time is muscle«* (as stated by the great Dutch cardiologist Paul Hugenholz in 1968), an early diagnosis is necessary to optimize the likelihood of successful reperfusion in patients with ischemic heart disease (IHD) (2).

According to the Joint European Society of Cardiology (ESC), American College of Cardiology Foundation (ACCF), American Heart Association (AHA) and World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction, an AMI can be diagnosed in the presence of an increase or decrease of cardiac biomarkers (preferably cardiac-specific troponins), with at least one measure exceeding the 99th percentile upper reference limit (URL), occurring in association with at least one of the following criteria: (a) symptoms suggestive of myocardial ischemia; (b) new or presumably new significant ST-segment-T wave (ST-T) variations, or new left bundle branch block (LBBB) in the ECG; (c) onset of pathological Q waves in the ECG; (d) evidence of recent loss of functional myocardial tissue or novel regional wall motion abnormalities; and (e) evidence of intracoronary thrombosis (3).

For ED patients with a resolved episode of chest pain and no electrocardiographic or biomarker abnormalities, the current clinical practice is based on observation in dedicated hospital areas along with performance of non-invasive testing, in accord with ACCF, AHA and ESC guidelines (4–6). The objective of this strategy is to minimize the risk of missing an acute coronary syndrome (ACS) and, most notably, an AMI. When ACS is diagnosed, the patient should be promptly admitted for appropriate management, whereas patients without a definite diagnosis should undergo further risk stratification with non-invasive tests such as exercise or pharmacological stress testing or coronary computed tomography angiography (CCTA), either prior to discharge or within 3 days when an ACS is excluded and the chest pain does not recur (7).

Zaključak: Podaci pokazuju da testiranje dipiridamolom stresom ne pokreće oslobađanje troponina kod pacijenata sa i bez inducibilne reverzibilne ishemijske.

Cljučne reči: troponin, stres-test, ehokardiografija, stres-echo, dipiridamol

To facilitate a more definitive evaluation while avoiding unnecessary hospital admission of patients with possible ACS and low-risk ACS, as well as the inappropriate discharge of patients with AMI without ST-segment elevation (STEMI), special units have been established, which are variously referred to as *»chest pain units«* (CPUs) or *»ED observation units«* (EDOUs). In those settings, critical pathways or protocols that have been designed for defining the presence or absence of myocardial ischemia (both unstable angina and non-ST-segment elevation MI) are typically used for establishing the optimal next step in patient care (e.g., admission, acute intervention). The goal is to establish the most suitable management after a limited amount of time, which is ideally set between 6 and 12 h, but may extend up to 24–36 h depending on local policies. The patient usually undergoes a predetermined observation period with serial cardiac biomarkers and ECG assessment. At the end of the observation period, the patient is re-evaluated and then generally undergoes functional cardiac testing (e.g., resting nuclear scan or echocardiography) and/or stress testing (e.g., treadmill, stress echocardiography, or stress nuclear testing) or non-invasive coronary imaging study (i.e., CCTA). With a structured use of CPUs, 46% reduction in hospital admissions after a median stay of 9.2 h has been reported (8, 9).

Stress echocardiography (stress echo) is one of the most frequently used provocative tests. The diagnostic endpoint for detection of myocardial ischemia is the induction of a transient worsening in regional function during stress. Stress echo provides similar diagnostic and prognostic accuracy as radionuclide stress perfusion imaging, but at a substantially lower cost, without environmental impact, and with no bio-hazards for patients and physicians. Among different stresses of comparable diagnostic and prognostic accuracy, dobutamine, adenosine and dipyridamole are the safest and simplest agents, as well as the most suitable for assessment of combined wall motion coronary flow reserve. In spite of its dependence on operator's training, stress echo can now be considered the best (i.e., the most cost- and risk-effective) imaging choice to achieve the still elusive target of sustainable cardiac imaging in the field of non-invasive diagnosis of CAD (10, 11).

As with other imaging techniques, the presence of inducible wall motion abnormalities is highly sensitive for significant CAD. Additionally, echocardiography provides information on valvular function and

contractile performance that may be useful in certain clinical circumstances. Few stress echo studies have been performed in a CPU setting, and none could demonstrate significant differences in hard cardiac events at follow-up (11–19). These studies, although failing to demonstrate differences in negative outcomes between patients with positive or negative test results, aimed at combined end points, including soft events, such as the number of elective revascularization procedures (mostly test-driven), recurrent angina, or CAD. Although previous reports, using non-highly sensitive (HS) troponin assays, did not show troponin release induced by stress echo (20), some concerns have subsequently emerged regarding possible drug-induced myocardial injury, with troponin release, linked to the procedure (21, 22). Therefore, this prospective study aimed to establish whether or not dipyridamole stress echo triggers release of troponin I (TnI) and T (TnT), which are universally considered as reliable biomarkers of ischemic and non-ischemic cardiocyte injury.

Materials and Methods

Study population

The study population included all consecutive patients admitted to the observation unit of the Academic Hospital of Parma (Parma, Italy) between 1st of July 2012, and 31st of December 2012 for further evaluation of chest pain at moderate-to-high probability (family history, previous ACS, hypertension, diabetes, smoking, hypercholesterolemia, renal failure). All patients, after exclusion of ongoing ACS during ED evaluation (i.e., no diagnostic ECG variation, and no rise-and-fall of troponin – troponin being determined with AccuTnI (Beckman Coulter Inc, Chaska, Minnesota, USA) – at time 0, 6 and 12 hours), were challenged with a provocative test consisting of both basal and pharmacological stress echocardiography using dipyridamole as a stressor agent.

The patients underwent dipyridamole-atropine echocardiography with adjunctive myocardial perfusion assessment between the end of the dipyridamole infusion (0.84 mg/kg/10 min) and the beginning of the atropine infusion. Consolidated endpoints and contraindications were used (11). The patients underwent both wall motion and myocardial perfusion studies using an iE33 echocardiograph with a S5 scan head (Philips Ultrasound, Bothell, Washington). Myocardial perfusion was assessed using SonoVue (Bracco Imaging Italia, s.r.l., Milan, Italy) as a continuous infusion (0.8 mL/min) with flash-replenishment low-mechanical index imaging. Regional wall motion analysis was evaluated at baseline and at peak stress using a semiquantitative wall motion score (i.e., normal, hypokinesia, and akinesia) on a 17-segment model of the left ventricle. Reversible wall motion abnormality was defined as either a new dyssynergy in a

region with normal function at rest or worsening of hypokinesia at rest in ≥ 1 segment. Normal perfusion after dipyridamole was considered present if the myocardium was fully replenished 1.5 to 2 seconds after the end of the flash impulse. The perfusion was defined as abnormal if the myocardium was not replenished after this time. A perfusion defect was scored as fixed or reversible according to its persistence at the recovery stage. The contrast-enhanced stress echocardiographic finding was defined as »abnormal« in the presence of ≥ 1 of the following in ≥ 1 myocardial segment: (1) reversible wall motion abnormality, (2) reversible myocardial perfusion defect, and (3) a fixed myocardial perfusion defect in patients without a previous AMI. The presence of a fixed perfusion defect at the site of a known previous AMI was considered a »normal« contrast-enhanced stress-echocardiographic result. All tests were evaluated off-line by 2 experienced echocardiographers.

Laboratory measurements

All subjects had blood sampling for biomarkers assessment immediately before the stress-test (T1), 1 h from conclusion of the test (T2), and 6 h afterwards (T3). Cardiospecific troponins were assessed in serum with one contemporary-sensitive and two HS methods, as follows: Beckman Coulter AccuTnI (Beckman Coulter Inc, Chaska, Minnesota, USA), which is characterized by the limit of detection (LoD) of 2.5 ng/L and the 99th percentile of the upper reference limit of 56 ng/L; the prototype Beckman Coulter HS-AccuTnI (Beckman Coulter Inc), which is characterized by the LoD of 2.5 ng/L and the 99th percentile of the upper reference limit of 32 ng/L; the Roche HS-TnT (Roche Diagnostics GmbH, Mannheim, Germany), which is characterized by the LoD of 2 ng/L and the 99th percentile of the upper reference limit of 15 ng/L (23).

Statistics

Data are shown as median and interquartile range (IQR). The correlation between methods was assessed with Spearman's correlation, whereas the significance of differences between time points was assessed by Mann–Whitney U test. The statistical analysis was performed using Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK). The study was carried out in accordance with the Declaration of Helsinki, under the terms of all relevant local legislation and informed consent was obtained from all the patients before performing the provocative test.

Results

Thirty-two subjects were finally included in this study. Sampling at T1 and T2 was available for all patients, whereas 7 of them were lost at follow-up (i.e., T3). The dipyridamole–atropine echocardiogra-

Table I Clinical and demographical data of the study population. Numerical variables are described as median and interquartile range (IQR), differences are evaluated by Mann–Whitney U test.

Dipyridamole stress echocardiography			
	Negative	Positive	p
n	25	7	
Age (years)	71 (60–77)	65 (57–73)	0.30
Gender (M/F)	16/9	4/3	0.74
Hypertension	10 (40%)	3 (43%)	0.89
Diabetes	11 (44%)	4 (57%)	0.54
Active smokers	7/22	2/7	0.98
GFR (mL/min/1.73 m ²)	82 (70–90)	85 (79–105)	0.31

GFR, Glomerular Filtration Rate (calculated with the Modification of Diet in Renal Disease equation)

phy test was positive in 7/32 patients (i.e., 22%). No difference was found for the main demographical and clinical data among patients with negative and positive stress tests (Table I). A highly significant correlation was found between AccuTnl and HS-AccuTnl values ($r=0.98$; $p<0.001$). A significant, but weaker correlation was also found between HS-TnT and AccuTnl ($r=0.39$; $p<0.01$), as well as between HS-TnT and HS-AccuTnl ($r=0.26$; $p=0.01$).

The main results of this study are synthesized in Table II. The concentration of AccuTnl, HS-AccuTnl and HS-TnT did not differ throughout the three time points. A trend towards higher values was observed at T3, but in no case this difference achieved statistical significance. The frequency of values exceeding the 99th URL was also similar throughout the study period. It is noteworthy, however, that although the limit

Table II Median and interquartile range (IQR) of AccuTnl, HS-AccuTnl and HS-TnT values in patients undergoing dipyridamole stress echocardiography before the test (T1), 1 h after conclusion of the test (T2), and 6 h afterwards (T3).

	T1	T2	p vs T1	T3	p vs T1
n	32	32		25	
AccuTnl					
Values (ng/L)	3.0 (0.0–9.6)	3.0 (0.4–8.6)	0.47	5.0 (0.0–13.0)	0.35
>99 th URL	3 (9%)	2 (6%)	0.20	2 (8%)	0.61
HS-AccuTnl					
Values (ng/L)	4.2 (3.0–9.7)	4.9 (2.8–12.8)	0.43	5.1 (3.0–12.3)	0.44
>99 th URL	3 (9%)	4 (13%)	0.35	2 (8%)	0.61
HS-TnT					
Values (ng/L)	10.0 (5.9–15.3)	10.2 (5.9–15.6)	0.47	11.7 (5.9–15.1)	0.44
>99 th URL	8 (25%)	9 (28%)	0.49	7 (28%)	0.50

HS, high-sensitivity; Tnl, troponin I; TnT, Troponin T; URL, Upper Reference Limit.

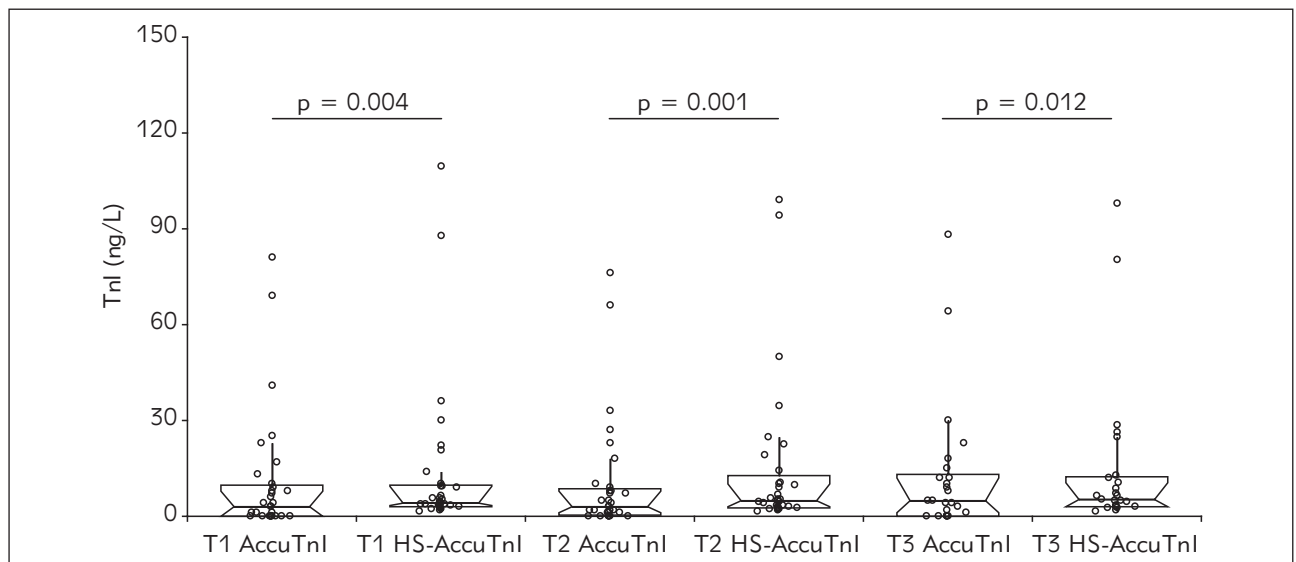


Figure 1 Median and interquartile range (IQR) of AccuTnl and Hs-AccuTnl values in patients undergoing dipyridamole stress echocardiography before the test (T1), 1 h after conclusion of the test (T2), and 6 h afterwards (T3).

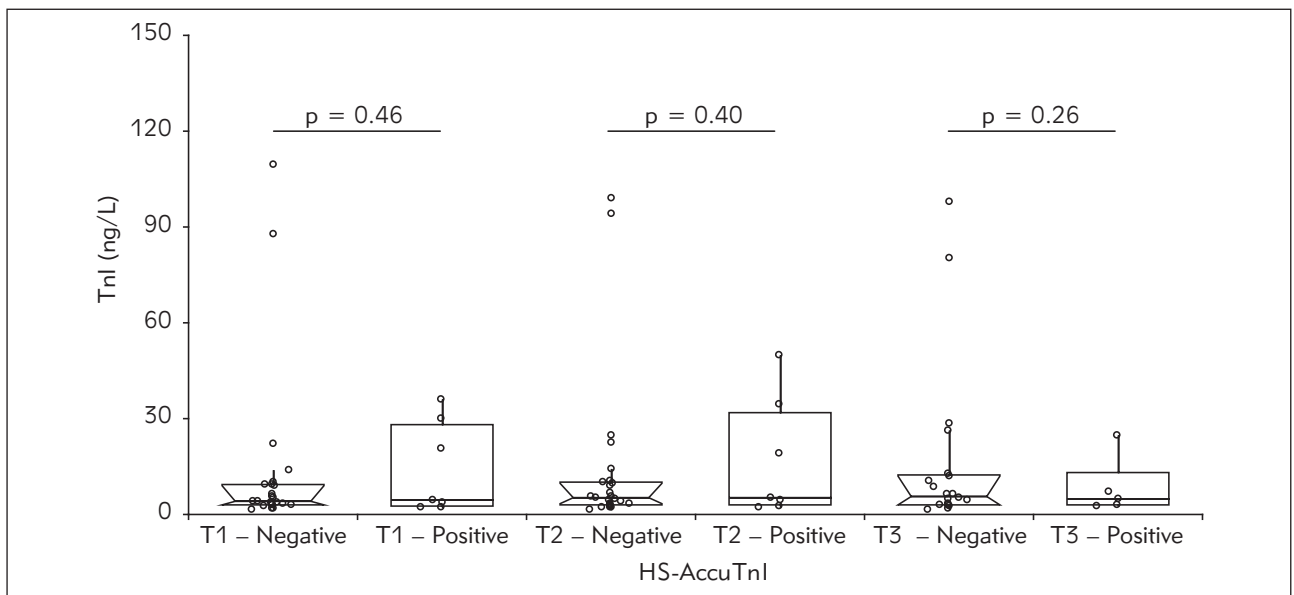


Figure 2 Median and interquartile range (IQR) of Hs-AccuTnI values in patients with positive and negative dipyridamole stress echocardiography before the test (T1), 1 h after conclusion of the test (T2), and 6 h afterwards (T3).

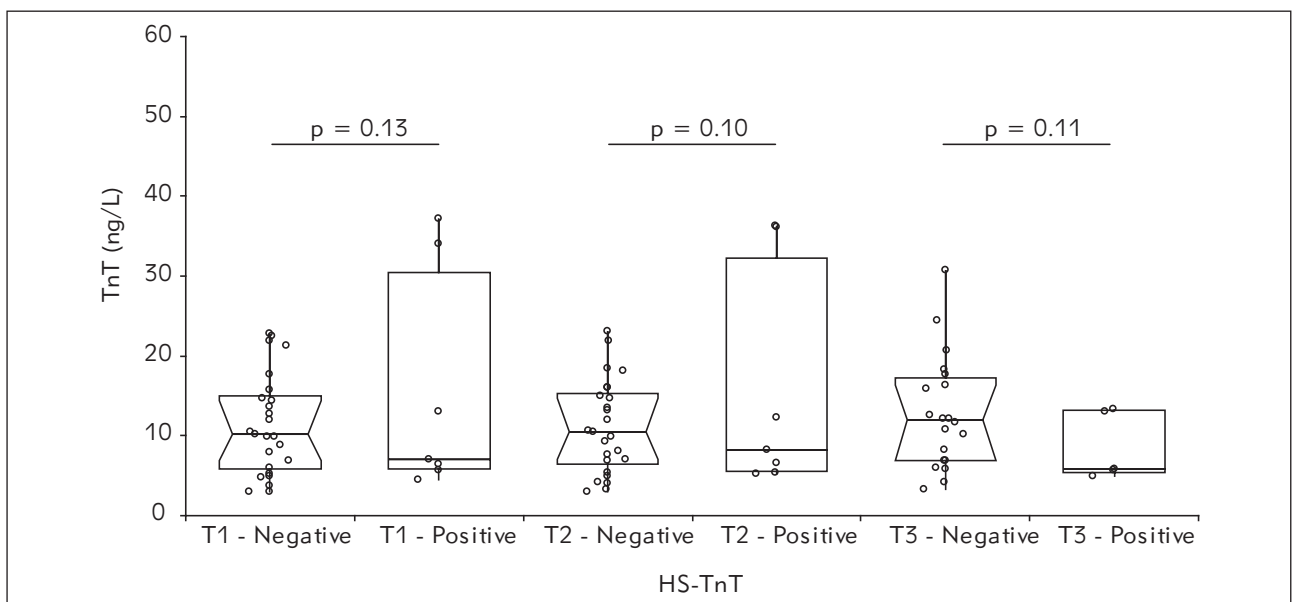


Figure 3 Median and interquartile range (IQR) of Hs-TnT values in patients with positive and negative dipyridamole stress echocardiography before the test (T1), 1 h after conclusion of the test (T2), and 6 h afterwards (T3).

was exceeded in less than 15% of cases with AccuTnI or HS-AccuTnI, the frequency of patients with HS-TnT values higher than the 99th URL was comprised between 25 and 28% across the three time points, i.e., it was nearly double that observed for AccuTnI and HS-AccuTnI. Interestingly, significantly increased values were found with HS-AccuTnI as compared with AccuTnI at the three time points, although the frequency of values above the respective 99th URL was globally comparable (T1: 9 vs 9%; $p=1.00$; T2: 13 vs 6%; $p=0.06$; 8 vs 8%; $p=1.00$) (Figure 1). The

values of HS troponin in patients with positive and negative stress test are shown in Figures 2 and 3. At no time point the concentration of either HS-TnI or HS-TnT was significantly different between patients with negative or positive stress test. Identical results were found with AccuTnI (data not shown).

Discussion

The remarkable technological advances achieved in laboratory technology and in the special field of

ACS diagnostics over the past decade (24) have allowed the production and commercialization of novel troponin methods, which are now characterized by substantially improved analytical sensitivity. Based on the availability of these HS immunoassays, several models for troponins release have been proposed, and it is now widely accepted that these proteins are released in many situations other than frank cardiomyocyte necrosis (25–27). The non-necrotic release of cardiac troponins has been attributed to leakage of free cytosolic pool, through a specific mechanism that involves the generation of the so-called cytoplasmatic »blebs«, containing a large amount of intracellular material. The smaller amount of Tnl compared to TnT in the cytosolic pool is the conceptual basis for those who hypothesize that HS-Tnl could be a superior biomarker to HS-TnT for detection of reversible myocardial injury (28). Although the cardiac stress test, either physical or pharmacologic, may hence represent a reliable model for assessing different aspects of reversible myocardial injury, the results of this study clearly attest that the measurement of neither HS-Tnl nor HS-TnT is able to identify patients with reversible myocardial ischemia (i.e., a »positive« stress test) in a clinically useful manner. Our results also corroborate the previous findings of Kurz et al. who, although limiting the measurement to a prototype HS-TnT, failed to observe any significant release of protein after physical exercise or pharmacologic stress with dipyridamole (29). At variance with these findings, Siriwardena et al. recently found a significant increase of HS-TnT after dobutamine stress echocardiography in 10 healthy volunteers and 16 patients with CAD. The increase was also more pronounced in subjects with a »positive« test. This study, however, differs from the present investigation for the use of another stressor drug (i.e., dobutamine vs. dipyridamole), while it is also noteworthy that the CAD patients with inducible ischemia had received the highest dobutamine doses (21). A few years ago, with the use of a different, experimental, HS assay for Tnl, the Thrombolysis In Myocardial Infarction (TIMI) study group found increased HS-Tnl concentrations after

stress testing in patients with reversible ischemia, but not in patients without ischemia. An adenosine-based stress test was used and the authors showed subtle elevations of Tnl, with a significant overlap of values between those with and without ischemia (22).

A limitation of our study is represented by the limited number of patients with reversible myocardial ischemia, so that further studies, including a larger study population, are advisable to confirm our findings.

Nevertheless, these results show that dipyridamole stress testing does not trigger release of Tnl (assessed with both a contemporary-sensitive and an experimental HS assay) or TnT, in patients with and without inducible reversible ischemia. Based on the available data, it thus seems plausible to put forward the hypothesis that dobutamine and dipyridamole may exert a different effect on the myocardium when used as stressor drugs, and that the latter agent may be safer and hence preferable. Indeed, due to the limited number of patients included in this study, larger investigations should be planned to unmask the mechanism(s) of troponin release during pharmacologic stress testing, most notably focusing on the different effects of different drugs. Another interesting finding of this study is the acceptable correlation found between the values of Tnl and TnT in our study population, which attest that the metabolism of these proteins may be very similar in patients undergoing stress testing, so that the tests may be used interchangeably in this setting. The substantial equivalence between the contemporary-sensitive and HS-Tnl immunoassays also suggests that the former method may be still suitable for routine assessment of patients undergoing stress testing.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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