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Effect of stress-induced reversible ischemia on serum concentrations of ischemia-modified albumin, natriuretic peptides and placental growth factor

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Summary *Objective* There is controversy whether new biomarkers are able to identify myocardial ischemia in the absence of myonecrosis. *Method* We measured NT-pro BNP, NT-pro ANP, ischemia-modified albumin (IMA) and placental growth factor (PlGF) in patients undergoing nuclear stress testing for suspected ischemic heart disease. A thallium scan was used for detection of reversible myocardial ischemia and cardiac troponin T (cTnT) for exclusion of stress-induced myonecrosis. Of 195 patients, 24 with reversible and 62 with no perfusion defect were included in the analysis. Plasma levels were measured before, 18 min and 4 h after stress testing. *Results* Of the 86 patients, 52 received an exercise stress and 34 dipyridamol. New myonecrosis indicated by cTnT could be excluded in all patients. Plasma levels of NT-pro BNP and NT-pro ANP before testing were significantly higher in patients who later developed reversible perfusion defects (NT-pro BNP 139.00 (58.25/367.01) pg/mL vs 327.45 (120.50/972.85) pg/mL, $p < 0.05$; NT-pro ANP 732.5 (470.0/1220.0) pg/mL vs 1470.0 (694.0/1910.0) pg/mL, $p < 0.05$). Plasma levels of NT-pro BNP, NT-pro ANP and PIGF did not change significantly after stress testing, IMA levels rose significantly after 4 h in patients with and without reversible perfusion defects. *Conclusion* The elevation of NT-pro BNP and NT-pro ANP at baseline may represent the cumulative effect of repeated bouts of myocardial ischemia. A single brief episode of provoced ischemia does not cause a significant increase of the measured biomarkers beside from IMA after exercise stress test potentially indicating skeletal muscle ischemia.

Key words
Myocardial ischemia – biochemical markers – troponin

Introduction

An increasing number of novel biomarkers have been reported to identify myocardial ischemia in advance or in the absence of myocardial necrosis. Among these, ischemia-modified albumin (IMA) and natriuretic peptides including B-type natriuretic peptide (BNP) or the N-terminal fragment (NT-pro BNP) are those studied most extensively. Several previous studies have demonstrated that IMA may increase in patients with both myocardial [24, 25] and skeletal muscle ischemia and after primary percutaneous intervention [1, 21, 29]. With respect to natriuretic peptides, experimental data have suggested that myocardial ischemia independently from myocardial stress, may stimulate the synthesis of proBNP

[18]. More recently, elevated levels of BNP and NT-pro BNP in the absence of myocardial infarction have been reported in patients with stable and unstable angina [13, 19] raising the hypothesis that levels of natriuretic peptides might increase after brief reversible ischemic episodes such as after protocol exercise testing [7, 8]. Placental growth factor (PIGF), a protein from the family of vascular growth factors, is claimed to indicate plaque instability at an even early stage. Recently it was found that PIGF levels were elevated in patients with stable angina suggesting a potential role of inducible myocardial ischemia [10]. However, the usefulness of all these biomarkers to indicate ischemia has not been demonstrated convincingly in clinical practice yet. The present study sought to evaluate the effects of reversible myocardial ischemia after nuclear stress testing on serum concentrations of IMA, NT-pro BNP, N-terminal atrial natriuretic peptide (NT-pro ANP) and PIGF.

Materials and methods

A total of 195 consecutive patients who underwent thallium scintigraphy (single-photon emission computed tomography – SPECT) for suspected significant coronary artery disease were studied. Patients underwent bicycle excercise testing using a standard exercise protocol or received pharmacological testing with dipyridamol at a dose of 42.72 mg (±7.61). The protocol was approved by the local ethical committee of the University of Heidelberg, and all patients gave informed consent prior to inclusion. Thallium was administered at peak stress and imaging was performed immediately and four hours after. A 17-segment myocardial model was used for semiquantitative analysis. Two nuclear cardiologists unaware of biomarker results categorized the images as having no perfusion defects, only reversible perfusion defects, a combination or fixed perfusion defects. Blood samples were obtained before, immediately after and 4 h after stress testing, placed on ice and processed within 30 min. Plasma aliquots were stored at –80 °C and thawed before analysis.

Biomarkers

cTnT was measured quantitatively using a one-step EIA based on electrochemiluminescence technology (3rd generation cTnT, Elecsys 2010, Roche, Mannheim, Germany). The lower detection limit of this assay is 0.01 µg/L with a recommended diagnostic threshold of 0.03 µg/L.

NT-pro BNP was measured using a commercially available sandwich immunoassay on a fully automated analyzer (NT-pro BNP ELECSYS 2010, Roche Diagnostics, Mannheim, Germany). The minimal detectable concentration is 5 ng/L, and the coefficient of variation is 5.7% at 64 pg/mL.

NT-pro ANP was measured using a competitive-binding radioimmunoassay with a magnetic solid phase technique using a rabbit-anti-rat proANP polyclonal serum, human proANP (1–30) from Peninsula Lab (Bachem Ltd, St. Helene, UK) and iodinated proANP (1–30) purified by HPLC for radiolabeling. The minimal detectable concentration is 105 pg/mL.

IMA was measured with the Albumin Cobalt Binding (ACB®) Test. The ACB Test (Ischemia Technologies, inc, Colorado, USA) is configured to run on the Roche/Hitachi Modular P instrument. Intra- and inter-assay precision were determined to be between CV 2.2–4.1% and CV 4.3–7.1%, respectively.

PIGF was measured by an enzyme-linked immunosorbent microtiter plate assay (R&D Systems, Wiesbaden, Germany). Total imprecision (expressed as coefficient of variation) for PIGF was 7.3%.

Statistical analysis

Plasma concentrations of cTnT, NT-pro ANP, NT-pro BNP, PlGF and IMA are described as median values with the corresponding interquartile range or as mean values with the corresponding standard error. The baseline characteristics of patient groups were compared using the Mann-Whitney U test or Students t-test for continuous variables and the chi-square test for categorical variables. The Kolmogorov-Smirnov test was used to test for normal distribution. For all analyses, a value of $P < 0.05$ was regarded as statistically significant. All statistical analyses were carried out using the SPSS software package version 12.01 (SPSS Inc, Chicago, IL, USA).

Results

Baseline characteristics

Among the entire cohort of 195 study participants 24 (12%) had reversible, 62 (32%) had no, 109 (56%) had either fixed or a combination of perfusion defects. All data and analyses on biomarkers presented in the following are confined to the 24 patients with reversible and to the 62 patients without perfusion defects. Of the patients with reversible perfusion defect, 16 (67%) were male (compared to 31 (50%), $p = 0.16$ without perfusion defect), mean

age was 70.72 years (vs 67.48, p = 0.26), body mass index (BMI) was 30 vs 27 kg/m^2, p = 0.12, 7 (29%) were smokers (17 (27%), p = 0.87), 18 (75%) had hypertension (49 (79%), p = 0.69), 15 (63%) hypercholesterolemia (43 (69%), p = 0.92), and 6 (25%) diabetes (15 (24%), p = 0.02).

Patients with reversible perfusion defects had more often a history of coronary artery disease, previous myocardial infarction, prior coronary intervention or coronary bypass graft surgery (83.3 vs 56.5%, p = 0.02) and underwent more frequently percutaneous coronary interventions (12.5% vs 1.6%, p = 0.04) after thallium scintigraphy.

Exercise testing

Of the final study group, 52 patients underwent bicycle exercise testing and 34 patients received dipyridamol stress testing. Detailed information on exercise parameters are given in Table 1.

Cardiac biomarkers before and after stress testing

Plasma levels of biomarkers were measured before, after a mean of 17.85 ± 11.14 min, and after a mean of 4.05 ± 0.64 h. Plasma concentrations of cTnT at baseline were below the lower limit of detection (0.01 μg/L) in all but 7 patients and did not increase significantly after stress testing. Increased plasma levels of cTnT in these 7 patients were related to prior acute myocardial infarction within 14 days before stress testing.

Baseline levels of NT-pro BNP and NT-pro ANP were significantly different in patients who later developed reversible perfusion defects. NT-pro BNP was significantly lower at baseline (median (25th/75th percentile): 139.00 pg/mL (58.25/367.01), n = 59) in patients without perfusion defects than in patients who developed reversible perfusion defects (median (25th/75th percentile): 327.45 pg/mL (120.50/972.85), n = 24, p < 0.05). NT-pro ANP was also significantly lower (median (25th/75th percentile): 732.5 pg/mL (470.0/1220.0), n = 36) in patients without perfusion defects as compared to patients with reversible perfusion defects (median (25th/75th percentile): 1470.0 pg/mL (694.0/1910.0), n = 10, p < 0.05). However, blood levels of NT-pro BNP and NT-pro ANP did not rise significantly at 18 min or at 4 h (Fig. 1 A–B). Relative changes of all cardiac biomarkers studied are shown in Table 2.

Plasma concentrations of IMA were comparable at baseline in patients without perfusion defects (n = 34) and in patients with reversible perfusion defects (n = 12). After a transient drop at 18 min IMA was raised significantly at 4 h. The increase of IMA occurred in patients with and without reversible perfusion defects (Fig. 1 C). Baseline levels of PIGF were comparable between patients who developed reversible perfusion defects (n = 20) compared to those without (n = 53). Blood levels did not increase significantly at 18 min or at 4 h after exercise test (Fig. 1 D).

Table 1 Stress test procedures

	Non-ischemia n = 62	Reversibel ischemia n = 24	P
Indication for stress test:			
■ Symptoms alone	8 (13%)	4 (17%)	0.18
■ Progress of known CAD	3 (37%)	1 (46%)	0.46
■ Angiography before exam	38 (61%)	18 (75%)	0.23
Type of stress test:			
■ Bicycle exercise	38 (61%)	14 (58%)	0.08
■ Dipyridamol	24 (39%)	10 (42%)	0.08
Test parameters:			
■ Duration (min):	7.74 (± 0.42)	8.75 (± 3.32)	0.23
– Bicycle exercise	5.21 (± 0.21)	5.00 (± 0.00)	0.85
– Dipyridamol			
■ Work load:			
– Peak Watts (bicycle)	109 (± 5)	125 (± 9)	0.15
– Total dose (mg Dipyridamol)	38.79 (± 1.38)	48.50 (± 7.03)	0.002
Clinical findings:			
■ Inducible angina	8 (13%)	4 (17%)	0.65
■ ST-depression > 0.15	10 (16%)	5 (21%)	0.61

CAD coronary artery disease, *CABG* coronary artery bypass graft, *PCI* percutaneous coronary intervention

Table 2 Relative changes of biomarkers before and after exercise-induced ischemia

	Baseline	After 18 minutes	After 4 hours
Biomarkers of cardiac function			
NT-pro BNP (pg/mL)			
No perfusion defect	139.00 (58.25/367.01)	161.00 (70.78/417.99)	168.95 (70.99/447.74)
Reversible perfusion defect	327.45[a] (120.50/972.85)	311.86 (126.66/816.41)	318.54 (142.58/1027.50)
NT-pro ANP (pg/mL)			
No perfusion defect	732.5 (470.0/1220.0)	810.5 (522.0/1295.0)	768.0 (490.3/1137.5)
Reversible perfusion defect	1470.0[a] (694.0/1910.0)	1410.0 (782.0/2040.0)	1345.0 (924.0/1770.0)
Biomarkers of ischemia			
IMA (U/mL)			
No perfusion defect	84.0[b] (80.0/93.0)	81.5[c] (69.0/91.0)	93.5 (84.0/106.0)
Reversible perfusion defect	82.5[d] (78.0/90.0)	71.0[e] (67.5/89.0)	96.5 (88.5/100.5)
Biomarkers of angiogenesis			
PlGF (ng/L)			
No perfusion defect	11.35 (5.98/20.25)	12.50 (6.77/23.50)	14.90 (6.40/23.40)
Reversible perfusion defect	15.15 (9.78/24.26)	16.08 (10.66/24.65)	17.99 (11.18/23.39)

cTNT cardiac troponin T, *NT-pro BNP* N-terminal pro-B-type natriuretic peptide, *NT-pro ANP* N-terminal atrial natriuretic peptide, *IMA* ischemia modified albumin, *PlGF* placental growth factor
[a] $p < 0.05$ for comparison between reversible perfusion defect versus no perfusion defect. All data are given as medians with corresponding 25th and 75th percentiles (Q1/Q3); [b] $p = 0.0070$ from baseline to 4 h; [c] $p < 0.0001$ from 18 min to 4 h; [d] $p = 0.0351$ from baseline to 4 h; [e] $p = 0.0130$ from 18 min to 4 h

Change of biomarker levels according to the type of exercise test

The baseline concentrations of all biomarkers were comparable in patients undergoing dynamic and in those undergoing pharmacological stress (data not shown). Concentrations did not change after stress except for IMA (Fig. 2).

IMA levels after 18 min were higher than baseline levels after pharmacological stress (n = 14, 97.0 U/mL (89.0/106.0) vs 89.0 U/mL (83.0/97.0), p = 0.1771) but lower after dynamic stress (n = 32; 75.0 U/mL (66.0/82.5) vs 83.0 U/mL (78.5/90.0), p = 0.0001). At 4 h IMA levels were significantly higher than levels at 18 min in patients who received dynamic (n = 32, 93.0 (86.0/100.5) vs 75.0 U/mL (66.0/82.5), p < 0,0001) compared to pharmacological stress (n = 14, 99.0 (85.0/108.0) vs 97.0 U/mL (89.0/106.0, p = 0.7304).

Discussion

Our study has three major findings. First, cTnT does not increase in response to reversible myocardial ischemia as detected with nuclear perfusion imaging. Second, baseline levels of NT-pro BNP and NT-pro ANP are significantly higher in patients in whom reversible myocardial ischemia is subsequently induced by stress testing. Third, there is a significant increase in blood levels of IMA after stress, regardless of the presence or absence of reversible myocardial ischemia.

In the present study, we used thallium scintigraphy for objective identification of reversible stress-induced perfusion defects. In order to exclude exercise-induced myocardial necrosis, cTnT was measured serially before and up to 24 h after stress testing. In all patients no significant increase of cTnT levels could be observed. Thus, our findings support experimental data showing that release of cTnT is exclusively restricted to irreversible myocyte necrosis [6]. Recently, small increases of cardiac troponins had been observed for a brief period after pulmonary embolism, after ultraendurance and endurance exercise [3, 11, 12, 16]. However, we cannot fully exclude the possibility that more severe reversible myocardial ischemia might have caused egress of soluble cardiac troponin due to membrane leakage since, in the present study, patients received standard protocol stress tests, which were stopped after onset of symptoms or occurrence of significant ST-segment depression.

Previous findings on IMA, natriuretic peptides and PlGF

Ischemia-modified albumin (IMA) has been proposed as a biochemical marker of cardiac ischemia [24, 25]. After initial enthusiasm, recent and more thorough work indicates that IMA is neither specific for myocardial ischemia nor for myocardial infarc-

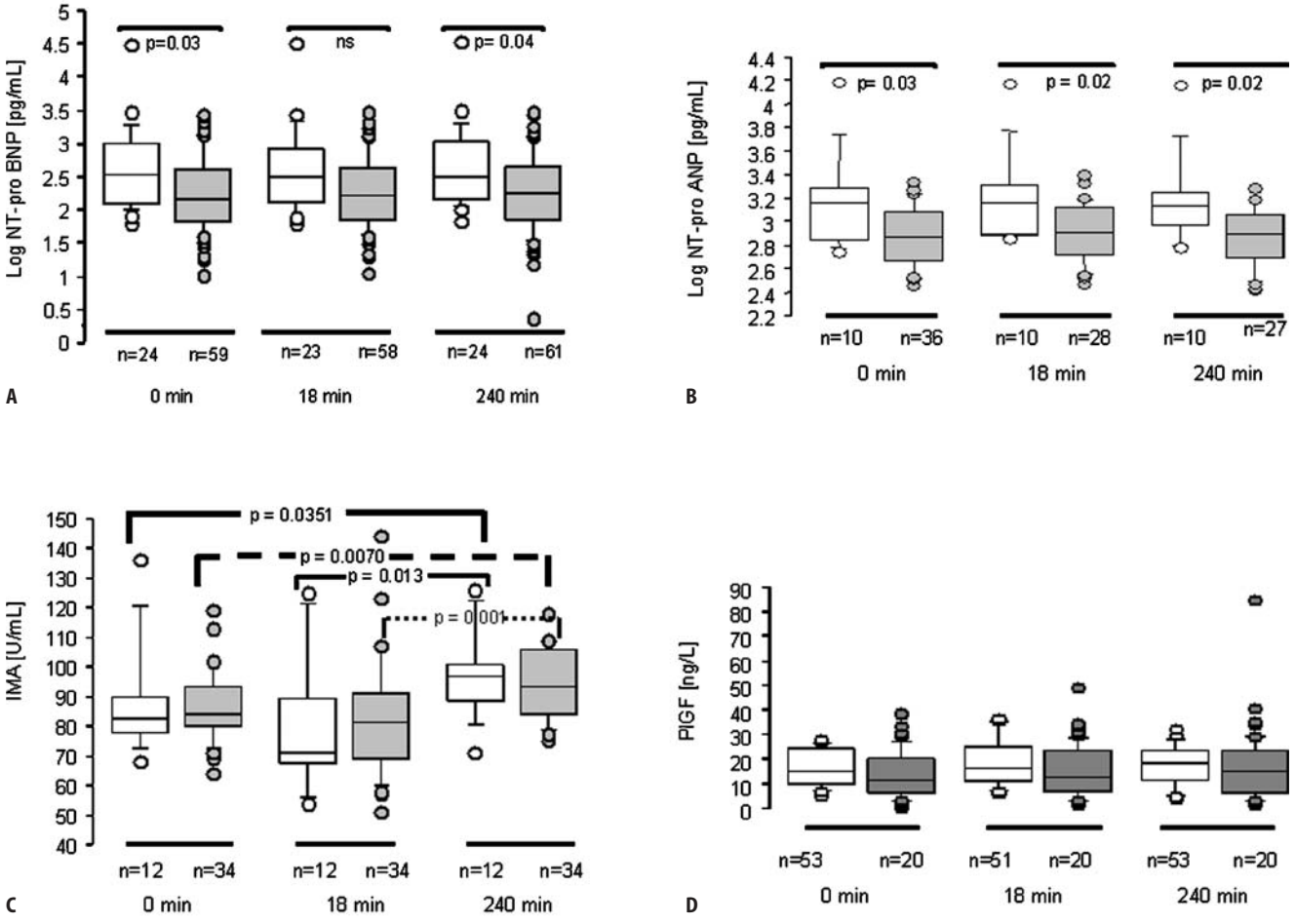

Fig. 1 Levels of NT-pro BNP (**A**), NT-pro ANP (**B**), IMA (**C**) and PlGF (**D**) before, 18 min and 4 h after protocol exercise test. White boxes represent patients with reversible perfusion defects and grey boxes represent patients without reperfusion defects. Box-plot bars indicate median, 25th and 75th percentile, error bars and extremes

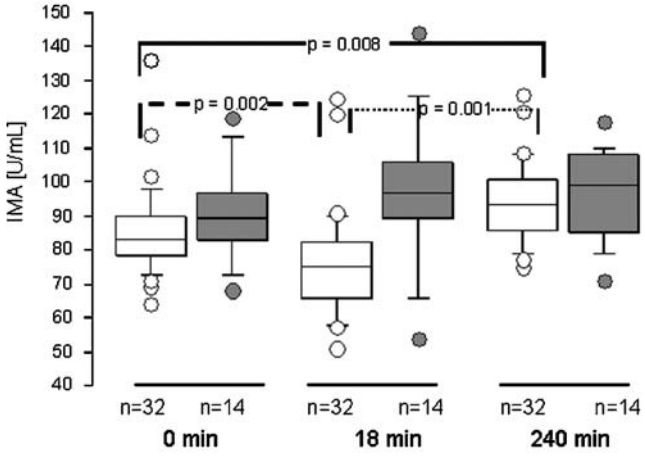

Fig. 2 Levels of IMA in patients undergoing bicycle ergometry (white boxes) or patients who received dipyridamol (grey boxes). Box-plot bars indicate median, 25th and 75th percentile, error bars and extremes

tion. Reduced cobalt-binding has also been reported after endurance exercise such as a marathon race [1], after radiofrequency catheter ablation [22], after skeletal muscle ischemia [29] or in patients with peripheral vascular disease [21]. Findings in patients with systemic sclerosis and experimental data indicate that serum albumin may be modified more likely by reperfusion after an ischemic event than by ischemia itself [2, 5]. Concordant with previous reports we were able to detect a significant increase of IMA levels 4 h after stress test both in patients with and without reversible perfusion defects. When split by the type of stress test, it could be demonstrated that patients with dynamic stress test had a more conspicous increase of IMA compared to patients with pharmacological test presumably because of peripheral skeletal muscle ischemia. However, the small size of the pharmacological group (n = 14 vs n = 32) could have also influenced that difference.

B-type natriuretic peptide and its N-terminal fragment (NT-pro BNP) are neurohormones synthesized and secreted from the ventricular myocardium after left ventricular wall stress [28]. Myocardial ischemia can cause transient LV systolic and diastolic dysfunction and thus an increase in BNP or NT-pro BNP levels. In addition, recent experimental data have demonstrated that myocardial ischemia may stimulate generation of BNP directly [4, 9, 23]. Increased BNP or NT-pro BNP levels have been found across the whole spectrum of acute coronary syndrome and in patients with stable angina [13, 19]. NT-pro BNP levels were closely associated with the number of significant lesions and correlated with the size of inducible ischemic myocardium in patients with stable angina. However, there was no significant increase in NT-pro BNP levels after exercise testing compared to baseline [26]. In that study, blood samples were taken only 15 min after termination of exercise presumably too short, given a half-life of 120 min for NT-pro BNP [20]. In line with our data baseline levels of BNP and NT-pro BNP were higher in patients with severe inducible myocardial ischemia as compared to those with mild-to-moderate or no inducible myocardial ischemia [23]. In contrast to our findings BNP values rose up to 20 to 30% immediately after exercise and returned to baseline after 4 h. The increase of NT-pro BNP was confined to patients with mild to moderate, whereas there was no significant increase in patients with severe myocardial ischemia. Careful interpretation of such small differences is needed given the high intra- and interindividual variation of BNP and NT-pro BNP [27]. In addition, it should be considered that NT-pro BNP levels may also increase after exercise in athletes and in normal subjects in absence of myocardial ischemia [15].

Sabatine et al. measured NT-pro ANP levels in 73 patients undergoing exercise testing [23]. Baseline levels of NT-pro ANP were significantly higher in patients with severe myocardial ischemia as compared to patients with only mild-to-moderate or without myocardial ischemia. A significant increase of NT-pro ANP immediately after termination of exercise with a subsequent decrease at 4 h was seen in both ischemic and non-ischemic patients. Interestingly, ANP is released in response to exercise-induced tachycardia by itself. Thus, it appears that ANP is less helpful to distinguish myocardial ischemia [14, 17]. Consistently, our study shows higher baseline NT-pro ANP levels in patients who later develop reversible perfusion defects on nuclear scan imaging. However, we found no significant increase in NT-pro ANP levels after termination of exercise or at 4 h.

Placental growth factor is a member of the vascular endothelial growth factor (VEGF) family. Recent findings have suggested a role of PIGF as a primary inflammatory instigator of atherosclerotic plaque instability. Heeschen et al. found that PIGF levels were elevated in patients with acute coronary syndromes providing prognostic information independent of cardiac troponin and sCD-40 ligand. PIGF levels were also higher in stable angina [10]. As an interesting finding, our data demonstrate that myocardial ischemia does not induce an increase of PlGF since there is neither a significant difference regarding the baseline PIGF level in ischemic patients compared to non-ischemic controls, nor do PIGF levels increase in response to myocardial ischemia or merely due to exercise. Discrepancies with the findings of Heeschen et al. may best be explained by differences with respect to study populations or severity of myocardial ischemia.

Limitations

Of the 86 included patients, only 15 had either angina or ECG changes during or after stress testing suggesting that the study population had only mild coronary heart disease at the time of examination. Compared to a thallium scan, a widely used method such as exercise ECG would have enlarged the study size, however, at the expense of sensitivity and objective identification of reversible perfusion defects. Thallium scan is able to identify myocardial ischemia earlier and is thus more sensitive than stress ECG.

Our data show some differences regarding baseline characteristics suggesting some selection bias. As patients were not allocated randomly, those who received pharmacologic testing stress were more sedentary and had more severe cardiac disease or non-cardiac comorbidities. In addition, those who experienced myocardial ischemia had a significantly longer duration of exercise or a higher total dose of dipyridamol. However, our primary intention was not to compare the effectiveness of different types of stress testing but rather to evaluate biomarker changes in response to the objective presence of reversible ischemia, regardless of how this was induced. It would have been interesting to evaluate the effects of dynamic vs pharmacological stress on biomarker response. However, the small sample size prohibited the breakdown of patient subgroups for a meaningful analysis.

Moreover, a healthy reference population without cardiac symptoms would have served better as a control group but exposure to potentially harmful radiation prohibited the inclusion of healthy individuals.

Blood was collected before exercise testing, after a mean of 18 min and 4 h after exercise. Therefore, any

increase of markers that occurred between 18 min and 4 h or beyond 4 h may have occurred undetected. Elevation of baseline levels of NT-pro BNP and NT-pro ANP in patients with inducible perfusion defects are highly likely due to previous exposure to a single or repeated bouts of ischemic events.

Conclusions

The study findings support the hypothesis that cTnT is a marker of irreversible myocyte death. To date, there are still no convincing data that reversible myocardial ischemia may cause any structural damage to the cell membrane or contractile apparatus resulting in a brief or prolonged release of soluble or structurally bound troponin. Our findings on natriuretic peptides, IMA and the vascular growth factor PIGF suggest that the elevation of NT-pro BNP and NT-pro ANP at rest may represent the cumulative effect of repeated bouts of myocardial ischemia causing upregulation of biosynthesis and increased secretion.

Finally, an increase of IMA after exercise stress test in patients without inducible myocardial ischemia indicates that skeletal muscle ischemia represents an important contributor and a major confounder that has to be considered for a meaningful interpretation of IMA results.

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