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# Autonomic Dysfunction is associated with High Mobility Group Box-1 Levels in Patients after Acute Myocardial Infarction

**Francesco Giallauria**[a,b,*], **Plinio Cirillo**[c,*], **Rosa Lucci**[a], **Mario Pacileo**[c], **Mariantonietta D'Agostino**[a], **Paola Maietta**[c], **Alessandra Vitelli**[a], **Massimo Chiariello**[c], and **Carlo Vigorito**[a]

[a] Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, University of Naples "Federico II", Naples, NA 80131, Italy

[b] National Institute on Aging, Longitudinal Studies Section - Clinical Research Branch at Harbor Hospital, 3001 S Hanover St, Baltimore, MD 21225, USA

[c] Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Division of Cardiology, University of Naples "Federico II", Naples, NA 80131, Italy

## Abstract

**Background—**High mobility group box-1 (HMGB1) protein, a critical mediator of inflammatory processes, is a novel predictor of adverse postinfarction clinical outcomes, being involved in the healing process after MI. Heart rate recovery (HRR), a marker of autonomic function defined as the fall in heart rate during the first minute after exercise, is a powerful predictor of mortality in postinfarction patients. The present study was designed to test the hypothesis that HMGB1 is associated with autonomic dysfunction in postinfarction patients.

**Methods—**Sixty-seven consecutive patients (mean age 59.3 years, 84% males) recovering from acute MI were included in the study protocol. All patients underwent Doppler-echocardiography, cardiopulmonary exercise and HMGB1 assay.

**Results—**HMGB1 levels were inversely correlated with peak oxygen consumption ($VO_{2peak}$) (r=-0.449, $P$<0.001), with left ventricular ejection fraction (LVEF) (r=-0.360, $P$=0.003), and with HRR (r=-0.387, $P$<0.001). In a linear regression analysis adjusted for multiple confounders, we found a significant inverse association between HMGB1 levels and HRR independent of age, gender, body mass index, $VO_{2peak}$, slope of increase in ventilation over carbon dioxide output ($VE/VCO_{2slope}$), and presence of diabetes (β=-0.377, p=0.034).

**Conclusions—**This study provided the first evidence for a significant association between increased HMGB1 levels and autonomic dysfunction expressed by post-exercise slower HRR in postinfarction patients. The prognostic implication of such association needs to be explored as well as whether HMGB1 could represent a valid marker for risk stratification either during the acute phase or long-term after MI.

Corresponding Author: Francesco Giallauria, MD, PhD. Cardiac Rehabilitation Unit, University of Naples "Federico II", via Pansini 5, 80131 Naples (Italy), giallauria@libero.it; Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, National Institutes of Health, 3001 S. Hanover Street, 21225 Baltimore, Maryland (USA), giallauriaf@mail.nih.gov.
*Drs Giallauria and Cirillo equally contributed to this article.

### Keywords

High Mobility Group Box-1; Autonomic Dysfunction; Heart Rate Recovery; Cardiac Remodeling; Myocardial Infarction; Left Ventricular Remodeling

## Introduction

Left ventricular (LV) remodeling after myocardial infarction (MI) is characterized by complex alterations in LV size and shape, continuing long after infarction healing, representing an important predictor of long-term mortality [1]. Inflammatory response and cytokine elaboration are integral components of the host response to tissue injury and play a particularly active role after MI [2].

High mobility group box-1 (HMGB1) is a ubiquitous nuclear protein, constitutively expressed in quiescent cells, where it is involved in several cellular functions, including determination of nucleosomal structure and stability, and binding of transcription factors to DNA sequences [3]. The evidence that HMGB1 is passively released from necrotic or damaged cells suggests HMGB1 as an effective stimulus triggering the inflammatory response [4].

Previous studies showed that loss of baroreflex sensitivity and increased sympathetic afferent activity determines an increase of total sympathetic nervous activity in heart failure [5]. The sympathetic nervous system negatively impacts the cardiovascular system in heart failure in several ways, including down-regulating beta1-receptors, exerting direct toxic effects on the myocardium, and contributing to myocardial remodeling and life-threatening arrhythmias [5].

Heart rate recovery (HRR), defined as the fall in heart rate during the first minute after exercise, is a marker of vagal tone which is a powerful predictor of mortality in patients with coronary artery disease (CAD) [6-8], independently from angiographic severity of CAD, left ventricular function, and exercise capacity [9].

Given the potential interplay between inflammatory response and autonomic dysfunction on cardiac remodeling, this study aimed at testing the hypothesis that higher HMGB1 is associated with autonomic dysfunction (as expressed by slower HRR) in patients after acute MI.

## Methods

### Study population

From October 2007 to November 2008, ninety-seven consecutive patients immediately after acute ST elevation myocardial infarction (first event) diagnosed according to American College of Cardiology/American Heart Association (ACC/AHA) guidelines [10] were screened for inclusion into the study. Patients were recruited at the Division of Cardiology, Department of Clinical Medicine, Cardiovascular and Immunological Sciences, University of Naples “Federico II”, Italy.

Patients with postinfarction residual myocardial ischemia, and pericarditis were excluded. Exclusion criteria were also age over 75 years, severe concomitant non-cardiac disease such as cancer, renal dysfunction (serum creatinine level over 3 mg/dl), and liver dysfunction (alanine aminotransferase/aspartate aminotransferase level >1.5 times the upper normal limit).

After exclusions, 67 postinfarction patients were enrolled into the study protocol. A number of assessments were performed in all postinfarction patients including: cardiovascular physical

examination; body mass index (BMI, ratio between the weight and the square of the height); 12-lead electrocardiography; Doppler-echocardiography; symptom-limited cardiopulmonary exercise stress test with HRR evaluation; blood chemistry and HMGB1 assay. Age-, gender- and BMI-matched subjects (n=20) with no cardiac disease were included in the study protocol as control group.

The study was conducted according to the guidelines of the Declaration of Helsinki, and institutional ethical committee of the University of Naples "Federico II" (Italy) approved the protocol. The purpose of the protocol was explained to each subject, and written informed consent was obtained before beginning the study.

## Cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPX) was performed 3-4 weeks after MI. All patients underwent incremental CPX on a bicycle ergometer (Vmax 29C, Sensormedics, Yorba Linda, California) and autonomic function was evaluated by heart rate recovery (HRR, the difference between heart rate at peak exercise and heart rate at first minute of the cool-down period), as previously described [11,12].

## Doppler-echocardiography

Doppler-echocardiography (Hewlett Packard Agilent Sonos 5500 phase-array scanner, Andover, MA, USA) was performed at a median of 1 day (range 0 to 3 days) after hospital admission as detailed elsewhere [13]. The Doppler-echocardiographic studies were all performed by the same physician who was blinded to the patient results of cardiopulmonary exercise stress testing and HMGB1 levels assay.

## High Mobility Group Box -1 assay

HMGB1 levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit assay according to the manufacturer's instructions (Shino-test Corporation, Kanagawa, Japan) [14]. Briefly, blood samples were collected from patients with acute MI within 3 hours after the hospital admission and immediately before thrombolysis or percutaneous transluminal coronary angioplasty (rescue or primary PTCA). After allowing the blood to coagulate, the serum was isolated by low-speed centrifugation at 4°C, and frozen/stored at -80°C until used to perform the ELISA test. The inter-assay as well as the intra-assay coefficient was <10%.

## Statistics

Sample size determination was based on a *t*-test assuming a normal distribution with non-equal variance with the mean and standard deviation for the experimental group (postinfarction patients) of HMGB1 levels equal to 15 ± 7 and 2 ± 1 ng/dl for the control group derived from a previous study, respectively (14). The required sample size was calculated to be 20 subjects per group to detect on the size of one SD with α value of 0.05 (two-sided) and power (1 – β) of 0.8. Data are presented as mean, standard deviation and percentage. For continuous variables, unpaired *t*-test was used to compare means. The bivariate correlations procedure was used to compute Pearson's correlation coefficients. Multiple linear regression analysis (stepwise method) was performed with HMGB1 levels as dependent variable and with age, gender, body mass index, $VO_{2peak}$, $VE/VCO_{2slope}$, LVEF, presence of diabetes and HRR as independent variables. In assessing the suitability of the data for a linear regression model, the collinearity diagnostics were evaluated. A subgroup analysis was performed aiming at testing the differences in HMGB1 levels and HRR between non-diabetic and diabetic patients. Statistical significance was indicated at the level $p<0.05$. Statistical tests were performed with SPSS software, version 15.0.0.

## Results

Clinical and demographic characteristics of the study population are shown in Table 1. Fifty-five percent of patients experienced anterior MI; 58% of the patients had a positive history of hypertension and 49% of dyslipidemia (Table 1). Thirty-two percent (n=16) of patients underwent primary PTCA (Table 1). Seventy-three percent of patients (n=49) underwent thrombolisis and rescue PTCA was performed in 16 out of 49 (32%) (Table 1). Sixty-eight percent (n=46) of patients were on angiotensin-converting enzyme inhibitor, 19% (n=13) of patients were on angiotensin receptor blocker; 65% (n=44) of patients were on beta-blockers and 43% (n=29) of patients were on statin therapy. HMGB1 levels, cardiopulmonary and Doppler-echocardiography data obtained in postinfarction patients were summarized in Table 2.

Control subjects (n=20) underwent medical history, physical examination, and complete blood chemistry. Each control was defined as age-, gender- and BMI- matched with postinfarction patients case when the differences between the case and control was less than 2 years and 1 $kg/m^2$ for age and BMI, respectively. Five subjects reported a positive history of hypertension whereas 3 subjects reported mild dyslipidemia. HMGB1 levels in postinfarction patients were significantly higher compared to controls (14.6 ± 6.7 *vs.* 2.9 ± 1.4 ng/ml, p<0.0001).

In postinfarction patients, bivariate correlation analysis showed that age was not correlated either with HMGB1 levels (r=0.180, *P*=0.144) or HRR (r=-0.135, *P*=0.277). Bivariate correlation analysis showed that HMGB1 levels were inversely correlated with $VO_{2peak}$ (r=-0.449, *P*<0.001), LVEF (r=-0.360, *P*=0.003), and HRR (r=-0.387, *P*<0.001) (Figure 1).

A subgroup analysis was performed in order to test the differences between diabetic (n = 13) and non-diabetic patients (n = 54). In postinfarction patients with diabetes, HMGB1 levels were significantly higher compared to postinfarction patients without diabetes (19.6 ± 7.0 *vs.* 13.4 ± 6.1 ng/ml, *P*=0.001). Postinfarction patients with diabetes showed slower HRR compared to postinfarction patients without diabetes (16.3 ± 4.0 *vs.* 20.4 ± 3.9 ng/ml, *P*=0.001). In diabetic patients, bivariate correlation analysis showed that HMGB1 levels were inversely correlated with HRR (r=-0.340, *P*=0.02).

We evaluated whether the association between HMGB1 levels and HRR was independent of age and gender. The association between HMGB1 levels and HRR was not influenced after adjusting for these two variables (Table 3, Model 1). Next, we attempted to identify potential mediators of the association between HMGB1 levels and HRR, by successively adding variables to the regression model. The association between HMGB1 levels and HRR remained unchanged after BMI was added to the model (Table 3, Model 2). Similarly, this association was not influenced by $VO_{2peak}$ (Table 3, Model 3), or by the addition of $VE/VCO_{2slope}$ (Table 3, Model 4). Finally, this association between HMGB1 levels and HRR remained unchanged after adjusting for LVEF (Table 3, Model 5), and presence of diabetes (Table 3, Model 6).

## Discussion

Several lines of evidence suggest that inflammatory response and autonomic function might potentially affect cardiac remodeling after MI. In this study, we showed a significant association between higher HMGB1 levels and slower HRR, a marker of autonomic dysfunction in postinfarction patients.

Previous studies showed that both exaggerate postinfarction inflammatory response [2, 15-17] and autonomic function [6-9] are associated with LV remodelling and poor clinical outcomes. An efficient postinfarction inflammatory response leads to an appropriate infarct-healing process and the formation of a scar with tensile strength, resulting in prevention of

infarct expansion. Despite the importance of the inflammatory response and healing process in postinfarction LV remodeling, the mechanisms that initiate and control these processes remain to be elucidated.

HMGB1 is quickly released extracellularly after ischemic injury, inducing the expression of pro-inflammatory cytokines and adhesion molecules as an inflammatory mediator [18]. A recent study showed that increased HMGB1 levels in patients with acute MI were associated with pump failure, cardiac rupture, and increased in-hospital cardiac death [19]. Interestingly, six months after MI, HMGB1 levels were positively correlated to serum C-reactive protein, suggesting that HMGB1 could be a predictor of adverse clinical outcomes and late-phase LV dysfunction after MI [19]. In addition, our research group found that increased HMGB1 levels in postinfarction patients were associated with impaired indices of cardiovascular functional capacity [14].

The pathophysiological role of HMGB1 in MI setting is controversial. Several lines of evidence have pointed out that HMGB1 might play a role in restoration of cardiac function after MI, probably by promoting stem cell recruitment and/or stimulating angiogenesis [20-22]. Exogenous administration of HMGB1 in the peri-infarcted LV might have therapeutic potential for the attenuation of LV remodeling in a permanent MI model through a mechanism that involves the activation of stem/progenitor cells [20-22]. In addition, the blockade of HMGB1 results in worsening of LV remodeling through impaired infarct healing and marked scar thinning, thus suggesting a key role of HMGB1 in the appropriate healing process and in preserving the structural integrity of the infarcted LV [19].

Conversely, other studies have underlined the negative effects exerted by HMGB1 on myocardial cells [23,24]. In particular, Tzeng et al. [23] recently found that HMGB1 might act as a novel myocardial depressant factor that may contribute to excessive and/or sustained inflammation and/or profound myocardial depression and myocardial collapse. Moreover, Hagiwara et al. [24] have demonstrated that this protein induces a negative inotropic effect in isolated rat hearts. Mechanisms by which HMGB1 might be involved in the negative effects on myocardial function have been partially explained with NF-κB, ERKs and PKCε suggested as potential cellular pathways [23,25].

It is clear that there is currently a disagreement regarding the role of HMGB1 in ischemic heart disease; the studies by Kitahara *et al.*[20] and Limana *et al.*[21] indicated that it is beneficial, whereas the study by Andrassy *et al.*[25] suggested the contrary. The reason for this discrepancy remains unclear at present; however, we should consider the differences in the experimental conditions of these studies. First, in the former two studies, permanent ligation of the left anterior descendent (LAD) coronary artery was performed, i.e. permanent MI was induced. In contrast, in the latter study, the heart was reperfused after a 30 min ligation of the LAD artery. There is a substantial difference between the pathophysiology of permanent MI and ischemia–reperfusion injury. Reperfusion is accompanied by the release of an excessive amount of oxygen-derived free radicals that cause reperfusion injury. The inflammatory responses, including neutrophils and macrophage infiltration, are much more severe in reperfusion injury than in infarction, suggesting that ischemia–reperfusion injury could enhance inflammatory cell activity.

The mechanisms by which HMGB1 might exert beneficial or deleterious effects on myocardium are not completely elucidated yet. It has been reported that HMGB1 administration is beneficial in low doses and detrimental in high doses, suggesting that the effects of HMGB1 are dose dependent [18]. In particular, HMGB1 secreted from the infarcted, necrotic myocardium might slightly increase its local concentrations, resulting in the proliferation of resident cardiac c-kit$^+$ stem cells and their differentiation into myocytes [20].

Conversely, in postinfarction patients who are quickly reperfused with PTCA or thrombolysis (i.e. our study population), increased recruitment of inflammatory cells such as neutrophils and macrophages due to ischemia followed by reperfusion, might significantly increase local HMGB1 levels with consequent detrimental effects on cardiac function. Therefore, HMGB1 might act as a double-edged sword in postinfarction inflammatory response and have bidirectional effects on LV remodeling depending on the site, extent, and timing of HMGB1 modulation [26].

Several observations might constitute the pathophysiological basis for understanding the prognostic effects of sympatho-vagal activity after MI [11,12]. The most obvious beneficial effect of cardiac vagal activity is to decrease cardiac work by reducing resting heart rate and contractility [27]. The combination of this reduction in contractility with a reduction in cardiac work and myocardial oxygen demand may be advantageous in the context of coronary artery disease and left ventricular dysfunction. In addition, stimulation of the vagus nerve inhibits sympathetic nerve activity via peripheral pre- [28] and post-synaptic interactions [29]. Myocardial exposure to high levels of noradrenalin results in β receptor-mediated cytotoxic effects and apoptosis as well as α receptor-mediated hypertrophic effects [30,31].

The pathophysiological mechanisms underlying the association between HMGB1 and HRR are unclear. Cross-sectional studies have suggested that autonomic function is related to inflammatory markers [32]. Experimental studies reported that vagal nerve stimulation could modulate inflammatory cytokines through the cholinergic anti-inflammatory pathway [33, 34], thus suggesting that HRR may be related to inflammatory markers through a cholinergic anti-inflammatory reflex. Interestingly, in patients with diabetes elevated levels of HMGB1 were significantly associated with slower HRR, which is strongly associated with cardiovascular outcome in these patients [35]. Indeed, the engagement of advanced glycation end (AGE) products with their receptor (RAGE) and subsequent signalling has been suggested as responsible of development of vascular complications in diabetic patients [36]. Moreover, in this patient population, HMGB1 can interact with RAGE or with Toll-like receptor on inflammatory cells thus activating inflammation-associated pathways which involve several pro-inflammatory cytokines [37]. This HMGB1-RAGE interaction might significantly contribute to faster progression of atherosclerosis in diabetic patients. Several studies have demonstrated the strong relationship existing between RAGE and diabetic neuropathy [38, 39]; thus, it could be plausible that the HMGB1-RAGE axis might be involved also in autonomic dysfunction as HRR impairment is observed in diabetic patients.

Given the recognized role of HMGB1 proteins in the healing process after MI and the beneficial effects of cardiac rehabilitation on both LV remodeling [40] and autonomic function [11,13], the results of a 6-month exercise-based cardiac rehabilitation trial currently ran in our unit would be helpful in determining whether exercise training modulates HMGB1 protein expression pattern and its relationship with some powerful indicators of cardiovascular function, such as cardiovascular capacity, autonomic dysfunction and LV remodeling after MI [41].

In conclusion, this study provides the first evidence of a significant association between inflammation (as expressed by increased HMGB1 levels) and autonomic dysfunction (as expressed by post-exercise lower HRR) in patients after MI. The prognostic implication of such association needs to be explored. Studies aimed at clarifying whether HMGB1 could represent a valid marker for risk stratification either during the acute phase or the follow-up phases in postinfarction patients are also strongly encouraged.

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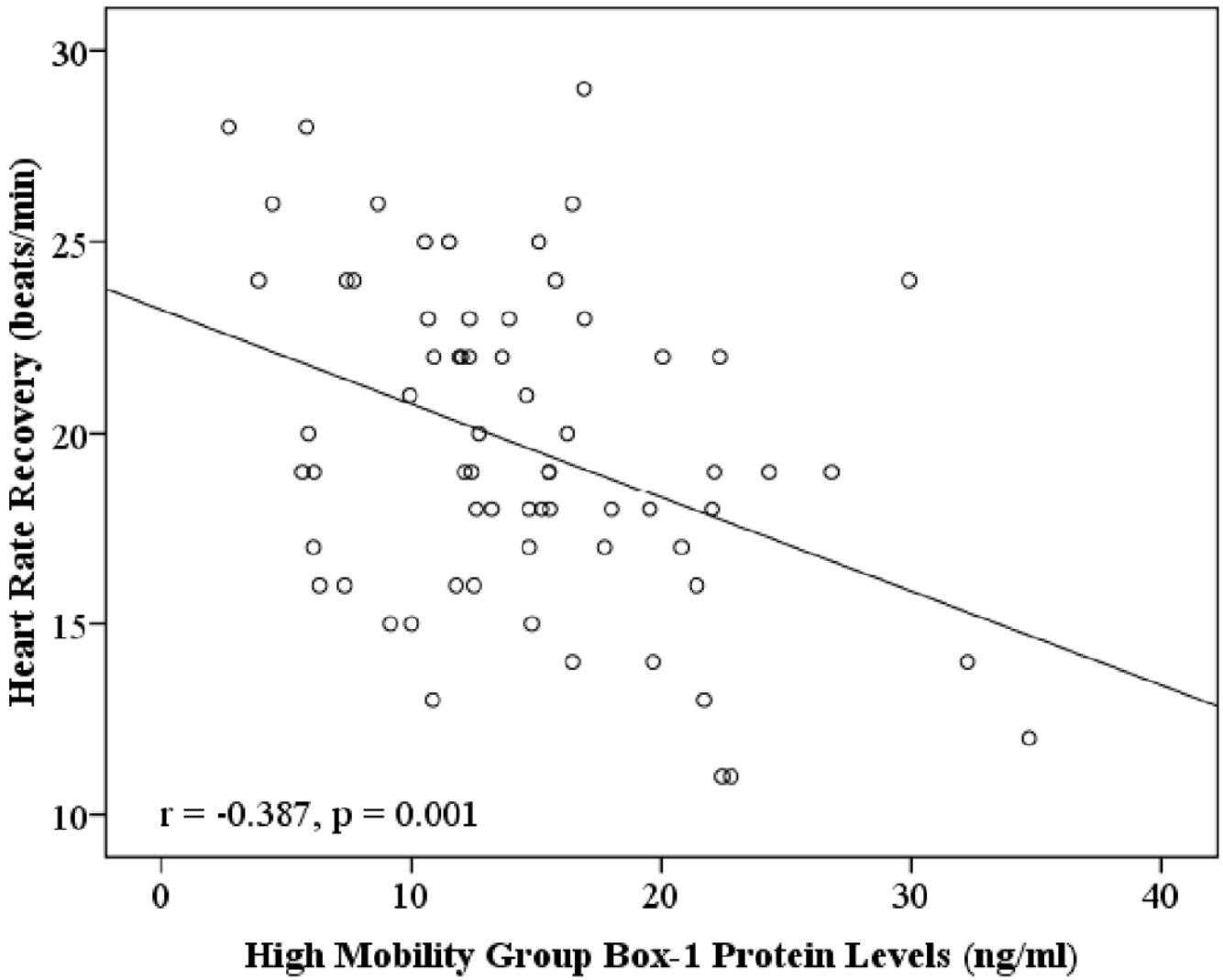


**Figure 1.**
The relationship between High Mobility Group Box-1 Protein Levels (ng/ml) and post-exercise Heart Rate Recovery (beats/min). The best fit line is shown.

## Table 1

Demographic and clinical characteristics of the study population (n = 67).

| | |
|---|---|
| **Age (years) (mean ± SD)** | 59.3 ± 8.5 |
| **Males (n, %)** | 56 (84) |
| **BMI (kg/m$^2$) (mean ± SD)** | 26.9 ± 2.9 |
| **Anterior MI (n, %)** | 39 (59) |
| **Inferior MI (n, %)** | 17 (25) |
| **Other MI locations (n, %)** | 11 (16) |
| **PTCA (primary) (n, %)** | 18 (27) |
| **Thrombolysis (n, %)** | 49 (73) |
| **PTCA (rescue) (n, %)** | 16 (32) |
| **Peak CK-MB (U/L)** | 277 ± 186 |
| **Peak Troponin I (ng/ml)** | 3.9 ± 16.5 |
| **Hypertension (n, %)** | 39 (58) |
| **Dyslipidemia (n, %)** | 33 (49) |
| **Diabetes (n, %)** | 13 (19) |

Data are expressed in mean ± standard deviation (SD) or percentage (%).

**Abbreviations:** BMI, body mass index; CK-MB, creatin kinase-MB; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty.

## Table 2

HMGB1 levels, Doppler-echocardiography and cardiopulmonary parameters of the study population (n = 67).

| | |
|---|---|
| **HMGB1 (ng/ml)** | 14.6 ± 6.7 |
| **LVIVSd (mm)** | 10.8 ± 1.9 |
| **LVPWTd (mm)** | 11.2 ± 2.2 |
| **LA dimension (mm)** | 39.6 ± 2.4 |
| **LVEDV (ml/m$^2$)** | 48.4 ± 6.9 |
| **LVESV (ml/m$^2$)** | 31.3 ± 7.4 |
| **LVEF (%)** | 47.7 ± 6.9 |
| **Peak E-wave velocity (cm/s)** | 59.5 ± 7.0 |
| **E/A ratio** | 0.94 ± 0.2 |
| **Systolic Blood Pressure (mmHg)** | 120 ± 9 |
| **Diastolic Blood Pressure (mmHg)** | 76 ± 5 |
| **Resting Heart Rate (beats/min)** | 77 ± 5 |
| **$VO_{2peak}$ (ml/kg/min)** | 14.4 ± 4.1 |
| **$VE/VCO_{2slope}$** | 31.9 ± 6.0 |
| **Heart Rate Recovery (beats/min)** | 20 ± 4 |

Data are expressed in mean ± standard deviation.

**Abbreviations:** HMGB1, high mobility group box-1 protein; LA, left atrium; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVIVSd, diastolic left ventricular interventricular septum; LVPWTd, left ventricular posterior wall thickness; $VO_{2peak}$, oxygen consumption at peak exercise; $VE/VCO_{2slope}$, slope of increase in ventilation over carbon dioxide output.

**Table 3**

**Multivariate Models Examining the Relationship between High Mobility Group Box-1 Protein Levels and Heart Rate Recovery**

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Covariates** | **B** | **SE** | **B** | **SE** | **B** | **SE** | **B** | **SE** | **B** | **SE** | **B** | **SE** |
| Age | 0.106 | 0.087 | 0.155 | 0.084 | 0.040 | 0.086 | 0.030 | 0.084 | 0.049 | 0.081 | 0.045 | 0.079 |
| Gender | 1.507 | 2.070 | 0.146 | 2.014 | -0.195 | 1.882 | 0.084 | 1.828 | 0.001 | 1.753 | 0.099 | 1.744 |
| BMI | … | … | 0.756 | 0.261† | 0.652 | 0.246† | 0.555 | 0.242* | 0.464 | 0.235* | 0.474 | 0.229* |
| $VO_{2peak}$ | … | … | … | … | -0.590 | 0.184† | -0.523 | 0.181† | -0.476 | 0.174† | -0.452 | 0.189† |
| $VE/VCO_{2slope}$ | … | … | … | … | … | … | 0.257 | 0.116* | 0.245 | 0.111* | 0.252 | 0.119* |
| LVEF | … | … | … | … | … | … | … | … | -0.235 | 0.093* | -0.229 | 0.101* |
| Diabetes | … | … | … | … | … | … | … | … | … | … | 0.355 | 0.264* |
| **Heart Rate Recovery** | **-0.575** | **0.182†** | **-0.558** | **0.172†** | **-0.496** | **0.162†** | **-0.427** | **0.160†** | **-0.396** | **0.154*** | **-0.377** | **0.156*** |

*p<0.05;

†p<0.01.

For each Model, β-coefficient (B) and Standard Error (SE) are given. Model 1 = age and gender; Model 2 = Model 1 + BMI; Model 3 = Model 2 + $VO_{2peak}$. Model 4 = Model 3 + $VE/VCO_{2slope}$; Model 5 = Model 4 + LVEF; Model 6 = Model 5 + presence of diabetes.

**Abbreviations:** BMI, body mass index; LVEF, left ventricular ejection fraction; $VO_{2peak}$, peak oxygen consumption; $VE/VCO_{2slope}$, slope of increase in ventilation over carbon dioxide output.