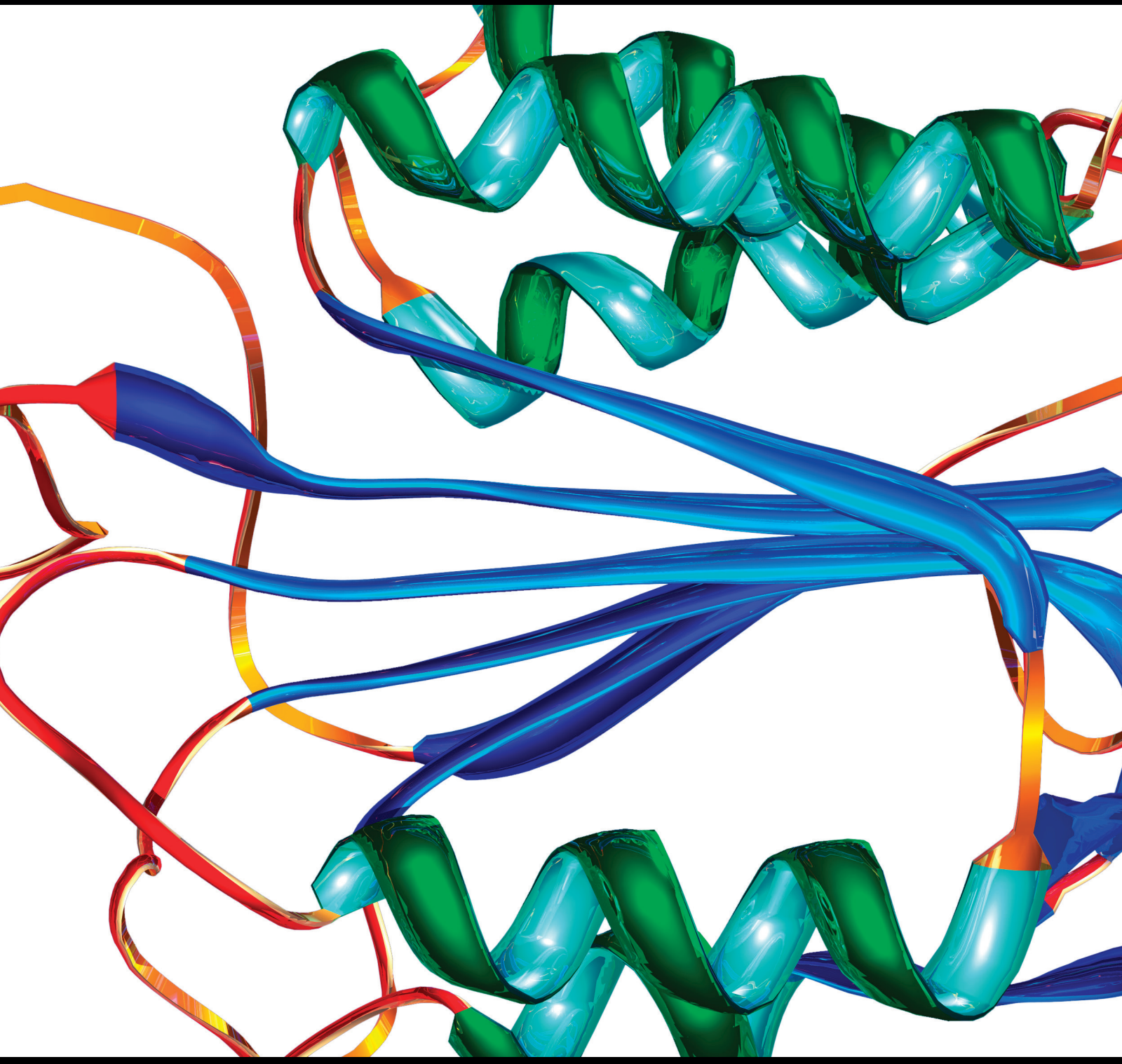
Disease Markers

# Cardiac Biomarkers

Guest Editors: Johannes Mair, Allan Jaffe, Fred Apple, and Bertil Lindahl



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# Editorial Board

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*Editorial*

# Cardiac Biomarkers

**Johannes Mair,[1] Allan Jaffe,[2] Fred Apple,[3] and Bertil Lindahl[4]**

[1]*Department of Internal Medicine III (Cardiology and Angiology), Innsbruck Medical University, 6020 Innsbruck, Austria*
[2]*Division of Cardiovascular Diseases and Internal Medicine, Mayo Clinic and Medical School, Rochester, MN 55905, USA*
[3]*Department of Laboratory Medicine and Pathology, Hennepin County Medical Center and University of Minnesota School of Medicine, Minneapolis, MN 55415, USA*
[4]*Department of Medical Sciences, Uppsala Clinical Research Center, Uppsala University, 75237 Uppsala, Sweden*

Correspondence should be addressed to Johannes Mair; johannes.mair@i-med.ac.at

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Cardiac biomarkers have evolved as essential tools in cardiology over the last 50 years, that is, for primary and secondary prevention, the diagnosis and management of acute myocardial infarction (AMI), and the diagnosis and risk stratification of heart failure (HF). We are beginning an era when it may be possible for biomarkers to direct treatment to optimize patient management. This is already the case with cardiac troponin (cTn) but should be the goal with all biomarkers. This special issue is a compilation of timely reviews and original articles on this topic.

More than 60 years ago in 1954 Karmen et al. [1] first reported that release of aspartate aminotransferase (AST), formerly glutamate oxaloacetate transaminase (GOT), from necrotic cardiac myocytes could be detected in the serum and could aid in the diagnosis of AMI. This initiated the era of enzymology in cardiology. In 1955 lactate dehydrogenase (LDH) was first published as a marker of AMI [2], and a direct enzymatic assay for $\alpha$-hydroxybutyrate dehydrogenase activity was later developed to increase cardiac specificity [3]. Subsequently an effective enzymatic assay for the quantification of creatine kinase (CK) activity was developed by Rosalki [4] and established this enzyme as the standard marker for the detection of muscle damage. CK remained the mainstay for AMI diagnosis for about 20 years. The development of immunoinhibition assays [5] for activity measurement of the more cardiac-specific isoenzyme CKMB on automated analyzers made this test and marker popular and widely used for many years. During the same period the Framingham study [6] established lipid concentrations and in particular cholesterol measured by enzymatic assays as risk factors for the development of cardiovascular diseases.

In the 1970s radioimmunoassays were developed and revolutionized laboratory medicine including AMI diagnosis. Immunoassays for myoglobin and CKMB were developed [7, 8]. Subsequently immunoassays were substantially improved by using monoclonal antibodies, and rapid immunoassays for measuring the so-called CKMB "mass" replaced CKMB activity measurements as the criterion standard for AMI diagnosis. Given the limitations of CKMB regarding cardiac specificity there was an intense search for potentially more specific cardiac damage markers in the 1980s. Finally only cardiac troponin I (cTnI) and cardiac troponin T (cTnT) turned out to be truly cardiac-specific and made the way from research to clinical routine use [9, 10]. This initiated the troponin era in the laboratory diagnosis of myocardial injury. Major interest was generated by studies indicating that cardiac troponins were useful for risk stratification and in identifying individuals most apt to benefit from an early invasive strategy in patients with acute coronary syndromes (ACS) [11–14], and a new key role for cardiac biomarkers was added to the traditional diagnostic role. Subsequent large clinical studies confirmed this prognostic role convincingly for both cTnT and cTnI, and also because of their additional outstanding cardiac specificity they were proposed as the new golden standard for the laboratory diagnosis of myocardial injury in a consensus statement published in 2000 [13]. cTnI and cTnT have remained criterion laboratory markers for the diagnosis of myocardial injury since then including in the subsequently published Universal Definitions of AMI [14]. During recent years the analytical sensitivities of cTnI and cTnT assays have improved remarkably to a degree that could not be expected at the beginning of the "immunoassay era," and recently, the

so-called "high-sensitivity" cTnT and cTnI assays have been introduced in routine clinical practice. These assays enable earlier detection of AMI obviating the need for other "early" necrosis markers, and with these assays cTn can be detected even in the majority of normal individuals [15].

About the same time in 1981, de Bold et al. made the observation that atrial myocardial extracts, when injected in rats, resulted in rapid and important natriuretic response [16]. This finally led to the discovery of the cardiac natriuretic peptide system. Natriuretic peptides are primarily synthesized in the heart and upregulated by myocardial stress mediated by volume, or pressure overload. B-type natriuretic peptide (BNP) and the N-terminal split product of its precursor hormone proBNP as well as N-terminal proatrial natriuretic peptide (ANP) turned out to be suitable laboratory markers for routine diagnosis and risk stratification of HF which opened totally new applications of laboratory testing in cardiology [17].

The 1990s was the golden era of cardiac biomarkers. The great clinical significance and economic impact of cardiac diseases triggered a huge research effort in the discovery of novel biomarkers for the diagnosis and risk stratification of ACS and HF as well as risk stratification in primary and secondary prevention. This led to the discovery of numerous biomarkers and the development of immunoassays which were also suitable for routine measurement. The main focus was on markers of coronary plaque formation, plaque destabilization, intracoronary thrombus formation (coagulation and platelet activation, reduced endogenous fibrinolytic activity), and markers of myocardial ischemia. However, the vast majority of these markers did not make the way from research to routine application due to analytical issues or because the clinical impact for risk stratification was limited because they did not add much to traditional risk factors and even in multimarker approach improved risk stratification and patient reclassification only very modestly. Critically, they did not lead to direct information about how to improve patient management.

During this period also genomic biomarkers entered the field and have been particularly popular in the last two decades. Almost all of the candidate-gene era genetic biomarkers of cardiovascular disease failed to be validated after an initial period of enthusiasm [18]. Rare variants may be potent but because they are rare, they do not identify large numbers of additional patients at risk. Common variants such as single genetic variants confer extremely small risks such that the usual way of calculating risk, such as ascertaining smoking habits and measuring blood pressure and cholesterol, is better than analyses for these commonly occurring variations in DNA sequences. Consequently, the current consensus is not to test for commonly occurring genetic variants with weak effects [19]. Currently, the study of microRNAs has also become a very popular area of research. MicroRNAs are small, regulatory, usually inhibitory noncoding RNAs which can be also detected in blood and could serve as biomarkers in cardiovascular disease [20]. However, it remains to be shown whether microRNAs will be relevant for routine cardiovascular diagnosis, risk stratification, or direction of therapy.

A possibility to potentially overcome limitations of single biomarkers is to combine them in multimarker panel testing to strengthen their clinical utility by combining the information of different aspects of the pathophysiology of cardiac diseases. This multimarker approach has been extensively studied but a breakthrough in this area has not yet occurred [21]. Future approaches could also combine proteins, lipids, metabolites, genetic markers, and imaging technologies. It is likely that, over time, panels will emerge as valuable in this area.

In summary, the role of biomarkers in cardiovascular diseases, such as AMI and HF, is very well established with cardiac troponin and natriuretic peptide testing as essential parts of patient evaluation. Despite major efforts in recent years, biomarkers for the prediction of coronary artery disease (CAD) and for risk stratification in stable and unstable CAD or the general population have not yet fulfilled their manifest promise [21]. The most established marker in this respect is high-sensitivity C-reactive protein which still remains controversial. It will be difficult for new cardiac biomarkers to substantially add to the information which can be obtained from the results of high-sensitivity cardiac troponin and natriuretic peptides.

## Disclosure

In the past year, Dr. Mair received minor consulting fees from Philips Health Care Incubator and Dr. Jaffe received research support and is a consultant for Siemens Medical Solutions Diagnostics and Beckman-Coulter. He is or has been a consultant to most of the diagnostic companies during recent years. Dr. Apple has received consulting fees from Instrumentation Laboratories and is a paid advisor to Philips Diagnostics. Dr. Lindahl has served as a consultant for Roche Diagnostics, Radiometer Medical, bioMérieux Clinical Diagnostics, Philips Healthcare, and Fiomi Diagnostics and also has received a research grant from Roche Diagnostics and lecture fees from Thermo-Fisher.

*Johannes Mair*
*Allan Jaffe*
*Fred Apple*
*Bertil Lindahl*

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*Research Article*

# Biomarkers of Hemodynamic Stress and Aortic Stiffness after STEMI: A Cross-Sectional Analysis

**Sebastian Johannes Reinstadler,[1] Hans-Josef Feistritzer,[1] Gert Klug,[1] Agnes Mayr,[2] Luc Huybrechts,[1] Angelika Hammerer-Lercher,[3] Johannes Mair,[1] Wolfgang-Michael Franz,[1] and Bernhard Metzler[1]**

[1] *University Clinic of Internal Medicine III, Cardiology and Angiology, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria*
[2] *Department of Radiology, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria*
[3] *Central Institute for Medical and Chemical Laboratory Diagnostics, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria*

Correspondence should be addressed to Bernhard Metzler; bernhard.metzler@uki.at

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*Aim*. Increased aortic stiffness might adversely affect cardiac structure, function, and perfusion. Release of biomarkers of hemodynamic stress is thought to be enhanced by these alterations. We aimed to evaluate the association between biomarkers of hemodynamic stress and aortic stiffness assessed at a chronic stage after ST-segment elevation myocardial infarction (STEMI). *Methods*. Fifty-four patients four months after STEMI were enrolled in this cross-sectional, single-center study. N-terminal pro–B-type natriuretic peptide (NT-proBNP), mid-regional pro–A-type natriuretic peptide (MR-proANP), and mid-regional proadrenomedullin (MR-proADM) levels were measured by established assays. Aortic stiffness was assessed by the measurement of pulse wave velocity using phase-contrast cardiovascular magnetic resonance. *Results*. NT-proBNP, MR-proANP, and MR-proADM concentrations were all correlated with aortic stiffness in univariate analysis ($r = 0.378$, $r = 0.425$, and $r = 0.532$; all $P < 0.005$, resp.). In multiple linear regression analysis, NT-proBNP ($\beta = 0.316$, $P = 0.005$) and MR-proADM ($\beta = 0.284$, $P < 0.020$) levels were associated with increased aortic stiffness independently of age, blood pressure, and renal function. NT-proBNP was the strongest predictor for high aortic stiffness (area under the curve: 0.82, 95% CI 0.67–0.96). *Conclusion*. At a chronic stage after STEMI, concentrations of biomarkers for hemodynamic stress, especially NT-proBNP, are positively correlated with aortic stiffness. These biomarkers might also be useful as predictors of high aortic stiffness after STEMI.

## 1. Introduction

In the last two decades, multiple studies have shown that increased arterial stiffness is independently associated with cardiovascular morbidity and mortality [1–4]. A recent meta-analysis convincingly confirmed that increased arterial stiffness is a strong predictor of morbidity and mortality in different patient cohorts with cardiovascular diseases [5]. Pathophysiologically, an increase in arterial stiffness is associated with (a) an increase in central pulse pressure, (b) an increase in cardiac afterload, and (c) reduced coronary perfusion due to a decrease in the central diastolic pressure [6, 7]. The current method of choice for the assessment of aortic stiffness is measurement of pulse wave velocity (PWV) [8]. Velocity-encoded, phase-contrast cardiovascular magnetic resonance (CMR) imaging provides a feasible and robust method to assess PWV [9–11].

Natriuretic peptides (NPs), such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and mid-regional pro–A-type natriuretic peptide (MR-proANP), are synthesised and secreted by cardiomyocytes [12]. Although myocyte stretch is thought to be the main trigger mechanism for

the production and secretion of these hormones, other important stimuli might be ventricular hypertrophy, inflammation, ischemia, or fibrosis [12]. Both emerged as important diagnostic and prognostic biomarkers in patients with acute myocardial infarction [13–16]. Mid-regional proadrenomedullin (MR-proADM) is a more stable fragment of adrenomedullin, a vasodilatory hormone, which is primarily secreted by the adrenal medulla [17]. Like natriuretic peptides, MR-proADM is a robust predictor of adverse outcome after acute myocardial infarction [14, 18]. We recently reported an independent association between aortic stiffness measured during the acute phase after ST-elevation myocardial infarction (STEMI) and NT-proBNP levels four months thereafter in 48 patients [19]. In a subgroup of 32 patients comparable correlations were observed for MR-proANP and MR-proADM. The relationship between these biomarkers and aortic stiffness assessed at a chronic stage after STEMI has not been investigated so far. Arterial stiffness might increase myocyte stretch, induce ventricular hypertrophy, and decrease myocardial perfusion, which are all potential trigger mechanisms for biomarker release. Therefore, we measured plasma levels of NT-proBNP, MR-proANP, and MR-proADM and correlated them to aortic stiffness assessed by CMR in a STEMI cohort four months after the index event.

## 2. Materials and Methods

*2.1. Study Population.* From November 2010 to March 2012, 54 eligible patients with first STEMI admitted to University Hospital of Innsbruck were included in this cross-sectional, single-centre study. STEMI was diagnosed according to the redefined ESC/ACC committee criteria [20]. Only patients treated by primary percutaneous coronary intervention within the first 24 hours after symptom onset were enrolled. Patients with a history of a previous myocardial infarction or angiographically proven coronary artery disease, an estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 $m^2$, Killip class > 2 at presentation, or contraindications to CMR analysis were excluded. Patient demographics were assessed by a detailed medical history/examination. The study was approved by the local ethics committee, and written informed consent was obtained from each participant.

*2.2. Blood Analysis.* Heparinized blood samples were collected from all patients 4 months following STEMI by peripheral venipuncture. Samples for NT-proBNP were promptly analysed at the central laboratory of the University Hospital of Innsbruck by personnel blinded to study data. MR-proANP and MR-proADM were measured in batches after storage at −80°C. Assays used for the determination of NT-proBNP, MR-proANP and MR-proADM have previously been described [19, 21]. Briefly, NT-proBNP concentrations were measured using a commercially available assay with an E170 instrument (proBNP II assay using monoclonal antibodies on a Modular, Roche Diagnostics, Vienna, Austria). The analytical limit of detection of NT-proBNP is 5 ng/L and the limit of quantification is 50 ng/L. The intra-assay coefficient of variations (CV) are 1.9% at a concentration of 64 ng/L and 1.2% at a concentration of 2105 ng/L, and the inter-assay CVs are 3.1% at a concentration of 46 ng/L and 2.7% at a concentration of 2170 ng/L according to the package insert. MR-proANP and MR-proADM were measured by fully automated fluorescence immunoassays (Kryptor, Thermo Fisher Scientific, B.R.A.H.M.S., Hennigsdorf, Germany). The analytical limit of detection of MR-proANP is 0.05 nmol/L and the limit of quantification 0.23 nmol/L. The limit of detection for the MR-proADM assay is 2.1 pmol/L and the limit of quantification is 4.5 pmol/L.

*2.3. Determination of Aortic Stiffness.* We used velocity-encoded, phase-contrast CMR imaging for the determination of PWV as described in detail previously [9, 11, 22]. In brief, all scans were performed with a 1.5 Tesla Magnetom Avanto scanner (Siemens, Erlangen, Germany) four months after STEMI. Two slices (128 phases per cardiac cycle) of retrospective ECG-triggered velocity-encoded phase-contrast sequences were set perpendicular to the ascending and abdominal aorta to measure through-plane flow. Spatial resolution was 1.3 × 1.3 × 8 mm. Velocity encoding was set to 150 cm/s and was adjusted in the case of aliasing artefacts. Aortic PWV was calculated as the mean propagation velocity between the ascending and abdominal aorta using the transit time method [11, 23]. Thereby, PWV is defined as the distance between the two aortic levels and the transit time between these sites.

*2.4. Statistical Analysis.* Statistical analysis was performed with SPSS Statistics 19 (IBM, Armonk, NY, USA) as well as MedCalc Version 13.1.2.0 (Ostend, Belgium). Kolmogorov-Smirnov test was applied to test for normal distribution. Results for continuous variables are all expressed as mean ± standard deviation or as median with interquartile range if not normally distributed. Pearson or Spearman-Rho correlations were performed as indicated. To determine whether there is an independent relation between PWV and biomarker levels multiple linear regression analysis was used. Nonnormally distributed variables were log-transformed for multiple regression analysis. Variables with a $P$ value < 0.05 in univariate analysis were included into the models. Differences in continuous variables between groups were determined by ANOVA test. To calculate the predictive utility of biomarkers (alone and in combination) for increased PWV, receiver operating characteristic (ROC) analysis was applied. For all data, a two-tailed $P$ value of <0.05 was considered to indicate statistical significance.

## 3. Results

Table 1 shows the characteristics of the patient cohort. All patients underwent a velocity-encoded, phase-contrast CMR scan for determination of PWV at 129 ± 20 days after STEMI. At that time 54 (100%) patients were on dual antiplatelet-(100% acetylsalicylic acid, 22% clopidogrel, 72% prasugrel, and 6% ticagrelor), 45 (83%) on beta-blocker-, 42 (78%) on angiotensin-converting enzyme inhibitor-, 6 (11%) on

Table 1

| Study population ($n = 54$) | Mean/median/number |
|---|---|
| Age, years | 59 ± 10 |
| Female, *n* (%) | 7 (13) |
| Body mass index, kg/m$^2$ | 27 ± 3 |
| Family history for AMI, *n* (%) | 12 (22) |
| Smoking status, *n* (%) | 25 (46) |
| Hypertension, *n* (%) | 44 (81) |
| Hyperlipidemia, *n* (%) | 36 (67) |
| Diabetes mellitus, *n* (%) | 5 (9) |
| Pain-to-balloon time, min | 261 (129–759) |
| Anterior STEMI, *n* (%) | 17 (32) |
| Culprit lesion, *n* (%) | |
| LAD | 16 (30) |
| LCX | 10 (18) |
| RCA | 28 (52) |
| Vessel disease, *n* (%) | |
| 1 | 24 (44) |
| 2 | 23 (43) |
| 3 | 7 (13) |
| Creatinine, mg/dL | 0.98 ± 0.15 |
| eGFR, mL/min/1.73 m$^2$ | 83 ± 15 |
| NT-proBNP, ng/L | 219 (119–412) |
| MR-proANP, pmol/L | 88 (68–128) |
| MR-proADM, nmol/L | 0.7 ± 0.2 |
| PWV, m/sec | 7.2 ± 2.0 |

AMI = acute myocardial infarction; STEMI = ST-segment elevation myocardial infarction; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro–B-type natriuretic peptide; MR-proANP = mid-regional pro–A-type natriuretic peptide; MR-proADM = mid-regional proadrenomedullin; PWV = pulse wave velocity.

angiotensin receptor antagonist-, and 53 (98%) on statin therapy. Mean PWV was 7.2 ± 2.0 m/sec. PWV did not differ significantly between men and women (7.1 ± 1.9 m/sec versus 7.7 ± 2.7 m/sec, $P = 0.468$, resp.). PWV was similar in patients with anterior STEMI and nonanterior STEMI ($P = 0.547$). There was no relationship between PWV and pain-to-balloon time ($r = 0.046$, $P = 0.740$). PWV was strongly correlated to patients' age ($r = 0.681$, $P < 0.001$). No significant correlation was found between PWV and blood pressure, body mass index, total cholesterol, creatinine, and estimated glomerular filtration rate (all $P > 0.05$). There was no significant difference in PWV between patients with or without diabetes ($P > 0.05$). Correlations between PWV and biomarkers of myocardial wall stress are shown in Figure 1. Importantly, log NT-proBNP, log MR-proANP, and MR-proADM were all significantly related to PWV ($r = 0.378$, $r = 0.425$, and $r = 0.532$; all $P < 0.005$, resp.). Partial correlation analysis revealed that NT-proBNP, log MR-proANP, and MR-proADM remained significantly correlated with PWV when adjusting for gender ($r = 0.367$, $r = 0.415$, and $r = 0.529$; all $P < 0.01$, resp.). In multivariate analysis, each marker was examined separately because of the close correlation between them (correlation coefficients between 0.5 and 0.7, $P < 0.001$). In the first model, age, eGFR, systolic blood pressure, diastolic blood pressure, and log NT-proBNP were taken as independent variables. This model revealed that NT-proBNP levels ($\beta = 0.316$, $P = 0.005$) and age ($\beta = 0.627$, $P < 0.001$) remained significantly associated with PWV ($R = 0.758$, $P < 0.001$). In the second model, age, eGFR, systolic blood pressure, and log MR-proANP were taken as independent variables. In this model age ($\beta = 0.641$, $P < 0.001$), but not MR-proANP ($\beta = 0.099$, $P = 0.411$), correlated with PWV ($R = 0.709$, $P < 0.001$). In the third model, age, eGFR, systolic blood pressure, and MR-proADM were taken as independent variables. Along with age ($\beta = 0.566$, $P < 0.001$), MR-proADM ($\beta = 0.284$, $P < 0.020$) remained significantly associated with PWV ($R = 0.741$, $P < 0.001$). Patients were also stratified in those with a PWV below ($n = 40$, 74%) and above ($n = 14$, 26%) the third quartile of PWV (=8.6 m/sec). The area under the curve (AUC) of NT-proBNP (0.82, 95% CI 0.67 to 0.96) with the optimal cut-off level of 270 ng/L revealed 86% sensitivity and 75% specificity for the prediction of an increased PWV. The AUCs of MR-proANP and MR-proADM for the prediction of an increased PWV (MR-proANP: 0.78, 95% CI 0.64 to 0.91; MR-proADM: 0.68, 95% CI 0.49 to 0.88) were lower compared with that of NT-proBNP, but the difference was not significant (NT-proBNP versus MR-proADM: $P = 0.185$; NT-proBNP versus MR-proANP: $P = 0.525$; MR-proADM versus MR-proANP: $P = 0.284$) (Figure 2). The combination of NT-proBNP with MR-proADM (AUC = 0.82, 95% CI 0.69 to 0.91), NT-proANP (AUC = 0.83, 95% CI 0.71 to 0.92), or MR-proADM and NT-proANP (AUC = 0.81, 95% CI 0.68 to 0.91) did not add significant prognostic information (all $P > 0.300$).

## 4. Discussion

In this cross-sectional study of fifty-four patients after first STEMI, we evaluated the association between a 4-month concentration of biomarkers for hemodynamic stress (NT-proBNP, MR-proANP, and MR-proADM) and aortic stiffness. We found significant, positive correlations between these biomarkers and CMR-derived aortic stiffness. Our results suggest that these biomarkers, especially NT-proBNP, might be useful for identifying patients with elevated aortic stiffness as well.

The association between arterial stiffness and cardiovascular risk has been well proven for a long time [5]. Increased aortic stiffness causes hemodynamic and myocardial wall stress, which might stimulate release of NT-proBNP, MR-proANP, and MR-proADM. In fact, an association between arterial stiffness and circulating levels of NT-proBNP has been described in the general population as well as in patients with various diseases [24–28]. This relationship was also observed for patients with stable coronary artery disease (CAD). Şahin et al. showed that in 411 consecutive patients with angiographically proven CAD NT-proBNP levels were independently associated with increased aortic stiffness [29]. Based on their results, the authors speculated that the NT-proBNP value might serve as a predictor of increased

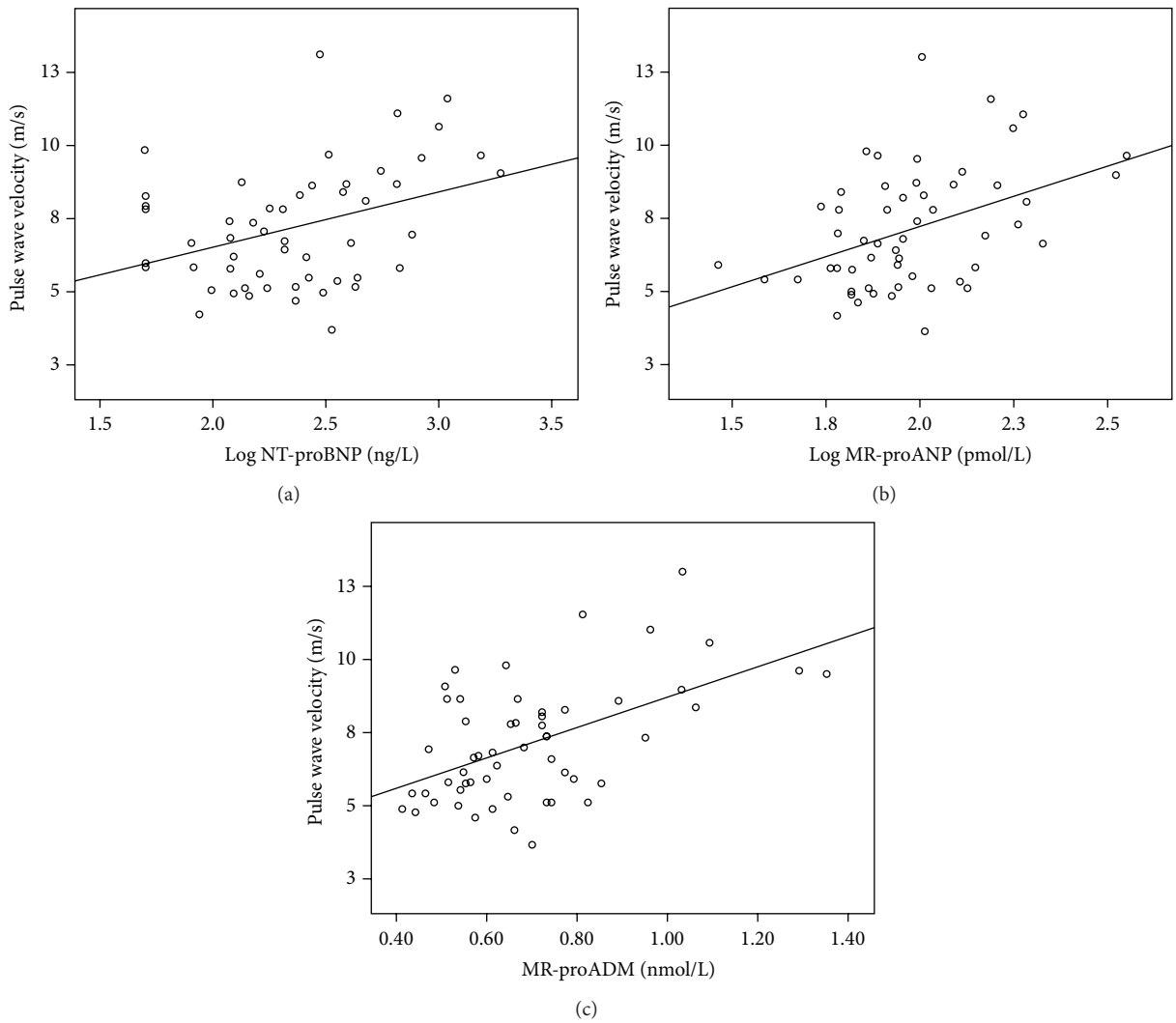


Figure 1: Univariate correlation between plasma NT-proBNP (a), MR-proANP (b), and MR-proADM (c) levels and aortic pulse wave velocity ($r = 0.378$, $r = 0.425$, and $r = 0.532$, resp.; all $P < 0.005$) in patients at a chronic stage after STEMI ($n = 54$).

aortic stiffness in patients with stable CAD. In contrast, there were only a few studies investigating the correlation between arterial stiffness and MR-proANP or MR-proADM [27, 30, 31]. These studies reported that MR-proANP and MR-proADM are also related to arterial stiffness. Recently, we have shown that aortic stiffness assessed in 48 patients during the acute phase after STEMI is associated with NT-proBNP levels four months thereafter [19]. In a subset of 32 patients, an association between aortic stiffness and MR-proANP as well as MR-proADM was also reported. In the present study, we show for the first time that concentrations of NT-proBNP, MR-proANP, and MR-proADM are significantly associated with increased aortic stiffness at the chronic phase after STEMI. In line with previous studies investigating other populations, the observed correlation coefficients were moderate to good. In ROC analysis, NT-proBNP performed best in predicting increased aortic stiffness defined as the upper quartile of PWV values. Although the sample size of the present study is relatively small, our results indicate that measurement of plasma NT-proBNP concentration at the chronic stage after STEMI could help identify patients with increased aortic stiffness. In this group of patients, assessment of aortic stiffness might be particularly useful for optimizing risk stratification at follow-up. Our results also indicate that aortic stiffening increases the release of NT-proBNP, MR-proANP, and MR-proADM presumably by increasing cardiac afterload also in patients at a chronic phase after STEMI. It is however important to note that there might be other trigger mechanisms than myocyte stretch leading to an increased secretion of this biomarkers in patients after STEMI. Left ventricular hypertrophy as well as fibrosis was shown to enhance gene expression of NPs [32]. Furthermore, stiffening of the aorta leads to an impaired cardiac perfusion [7]. Since myocardial ischemia can also induce production as well as secretion of NPs this might be a further explanation for the observed associations.

Of note, there is enough evidence to show that central arteries stiffen with advancing age [9, 33, 34]. In addition,

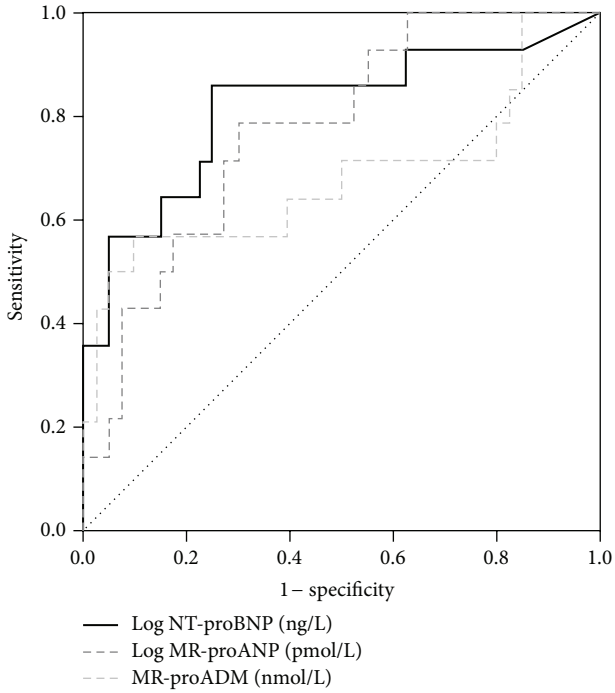


Figure 2: ROC curves for the predictive value of NT-proBNP, MR-proADM, and MR-proANP for increased PWV (=8.6 m/sec, $n = 14$, 26%). The AUCs were as follows: NT-proBNP: 0.82, 95% CI 0.67 to 0.96; MR-proANP: 0.78, 95% CI 0.64 to 0.91; MR-proADM: 0.68, 95% CI 0.49 to 0.88. There was no significant difference between AUCs of each biomarker (all $P > 0.05$).

natriuretic peptides and MR-proADM are also related to age [24, 35]. Our findings confirm these data in patients at a chronic stage after STEMI, since age was closely correlated with aortic stiffness and only moderately with biomarker levels. Other major confounders are blood pressure and renal function [36, 37]. Importantly, in multiple linear regression analysis, plasma levels of NT-proBNP and MR-proADM remained significantly related to aortic stiffness after correction for age, blood pressure, and renal function. By contrast, MR-proANP concentrations were not independently related to PWV in multiple linear regression analysis. A potential explanation for this finding might be that the stimuli for ANP and BNP release might be different, especially in patients with ischemic heart disease [12, 38]. Further studies are necessary to verify this possible explanation.

The cross-sectional design of the study precludes conclusions on a potential causal and temporal relationship between aortic stiffness and the reported biomarkers. Mean PWV was similar to that of mean PWV reported in a previous study assessing PWV in the acute phase after STEMI [19]. Hence, long-term longitudinal investigations with a large number of patients are needed to clarify this question.

A major limitation of this work is the relative small sample size and the fact that females represent only 13% of the study cohort. In partial correlation analysis, the association between biomarkers and PWV remained significant when considering gender. Nevertheless, conclusions regarding gender related differences cannot be drawn from this study. Investigations with a higher patient number as well as a higher percentage of women are necessary to confirm our data and to characterize possible gender differences in detail.

## 5. Conclusions

This is the first study showing that, at a chronic stage after STEMI, levels of NT-proBNP, MR-proANP, and MR-proADM are significantly associated with increased aortic stiffness. Among these biomarkers, especially NT-proBNP might be useful for predicting high aortic stiffness after STEMI. Larger investigations are needed to confirm the results of this study.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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*Research Article*

# Diagnostic Implications of an Elevated Troponin in the Emergency Department

**Maame Yaa Yiadom,[1] Petr Jarolim,[2] Cathy Jenkins,[3] Stacy E. F. Melanson,[2] Michael Conrad,[2] and Joshua M. Kosowsky[4]**

[1]*Department of Emergency Medicine, Vanderbilt University, Nashville, TN 37232, USA*
[2]*Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA*
[3]*Department of Biostatistics, Vanderbilt University, Nashville, TN 37203, USA*
[4]*Department of Emergency Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA*

Correspondence should be addressed to Maame Yaa Yiadom; myiadom@gmail.com

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*Objective*. To determine the proportion of initial troponin (cTn) elevations associated with Type I MI versus other cardiovascular and noncardiovascular diagnoses in an emergency department (ED) and whether or not a relationship exists between the cTn level and the likelihood of Type I MI. *Background*. In the ED, cTn is used as a screening test for myocardial injury. However, the differential diagnosis for an initial positive cTn result is not clear. *Methods*. Hospital medical records were retrospectively reviewed for visits associated with an initial positive troponin I-ultra (cTnI), ≥0.05 $\mu$g/L. Elevated cTnI levels were stratified into low (0.05–0.09), medium (0.1–0.99), or high (≥1.0). Discharge diagnoses were classified into 3 diagnostic groups (Type I MI, other cardiovascular, or noncardiovascular). *Results*. Of 23,731 ED visits, 4,928 (21%) had cTnI testing. Of those tested, 16.3% had initial cTnI ≥0.05. Among those with elevated cTn, 11% were classified as Type I MI, 34% had other cardiovascular diagnoses, and 55% had a noncardiovascular diagnosis. Type I MI was more common with high cTnI levels (41% incidence) than among subjects with medium (9%) or low (6%). *Conclusion*. A positive cTn is most likely a noncardiovascular diagnosis, but Type I MI is far more common with cTnI levels ≥1.0.

## 1. Introduction

Myocardial infarction (MI) in emergency department (ED) patients is a frequently suspected but infrequently made diagnosis. In the ED patient population a troponin serum assay (cTn) is used as a generic screen for myocardial injury [1, 2]. Serum cTn testing is the biomarker of choice to test for myocardial infarction, one of the more critical conditions causing myocardial injury. An elevated troponin has been associated with increased morbidity and mortality. So this generally prompts a continued evaluation in hospital [3]. The biochemical specificity of contemporary cardiac troponin assays, a troponin I (cTnI) upon initial serum testing in the ED, is greater than 90% for myocardial injury [4, 5]. This permits the early identification of patients with likely myocardial injury. A 2008 study by Keller et al. suggests that the diagnosis of acute myocardial infarction can be made early for many patients with an initial early elevated cTn level in the right clinical context. However, the likelihood that an initial elevated cTnI specifically represents Type I MI is less clear. Troponin based diagnostic decision-making primarily pertains to patients with potential non-ST-elevation MI (NSTEMI), since the diagnosis of STEMI is made by EKG. However, troponin testing still has a role in differentiating cases of MI from those where ST elevations on EKG may be attributed to other causes.

Even among causes of MI, there are several other mechanisms of injury to be noted. In these cases, acute management focuses on treating the underlying causes, rather than medication or procedural coronary intervention. This diversity was recognized within the universal definition of myocardial infarction as classified by the international consortium of

Table 1: Types of myocardial infarction (MI).

| Type | Definition |
|---|---|
| I | Infarction due to ischemia from a primary coronary event such as atherosclerotic plaque rupture and thrombus formation, arterial wall erosion, fissuring, or dissection |
| II | Infarction secondary to ischemia from either increased oxygen demand or decreased supply (coronary artery spasm, hypotension, hypertension, anemia, and dysrhythmia) |
| III | Sudden cardiac death/arrest with symptoms suggestive of STEMI or thrombus in a coronary artery found on angiography or autopsy |
| IVa | Infarction resulting from percutaneous coronary intervention (PCI) |
| IVb | Infarction from stent thrombosis |
| V | Infarction due to ischemia related to coronary artery bypass grafting |

cardiology associations. (see Table 1) [6]. Type I MI was independently distinguished from other mechanisms of acute myocardial infarction (AMI), as myocardial cell loss from ischemia caused by acute atherosclerotic plaque rupture with thrombus formation within a coronary artery lumen, fissuring, or dissection (i.e., acute coronary syndrome).

The loss of myocardium occurs on the order of minutes; thus a diagnosis of Type I MI prompts an acute shift in focus toward early antiplatelet and anticoagulant therapy with the possibility of urgent percutaneous coronary intervention (PCI). Little epidemiological data exists to guide practitioners in gauging the suspicion for Type I MI, amongst other causes, when the cTn is elevated in the context of a broad differential diagnosis. This situation is typical in the ED. The first objective of this study is to assess the frequency and implications of the diagnosis of Type I MI in an undifferentiated ED patient population. In addition, it is commonly assumed that the higher the cTn elevation, the greater the likelihood of Type I MI. A 2001 substudy by Lindahl et al. [7] suggested that this was the case with a cTnT assay. We challenge this hypothesis using a cTnI assay.

## 2. Materials and Methods

*2.1. Study Population.* This study was performed at a tertiary care academic medical and trauma center with 56,000 adult ED patient visits a year using a sensitive contemporary troponin biomarker for the evaluation of MI. Prior to the initiation of this retrospective chart review, it was approved by the hospital human research committee. The study period was March 1–July 31, 2007. The study population included all patients ≥18 years of age with a positive (≥0.05 $\mu$g/L) initial ultrasensitive cTnI (TnI-Ultra, Siemens Healthcare Diagnostics, Tarrytown, New York) [8, 9] during their ED evaluation. Patients presenting in cardiac arrest were excluded. Typical practice in the ED at this time was to test appropriate patients for a cTn elevation upon initial evaluation and then 6 hours after presentation. Patients with a cTn elevation at first measurement were included. Results were obtained from the laboratory information system and merged, with the associated patient data from the electronic medical record system, in Microsoft Excel.

*2.2. Adjudication of the Final Diagnosis.* The final hospital discharge diagnosis was confirmed through a review of each patient's ED and in-hospital record by a senior emergency medicine attending physician. Patients were categorized into diagnostic groups as either Type I MI (on the basis of care consistent with MI management including urgent revascularization, sustained antithrombotic therapy, or percutaneous coronary intervention during the hospital course), other cardiovascular diagnoses (including dysrhythmias, pulmonary embolism, and congestive heart failure), or a noncardiovascular diagnosis. In addition, deaths and the incidence of urgent revascularization were noted.

*2.3. Analysis of Troponin Results.* TnI results were stratified as low (0.05–0.09 $\mu$g/L), medium (0.1–0.99 $\mu$g/L), and high (≥1.0 $\mu$g/L). Frequencies of primary diagnoses were determined by troponin strata, major diagnostic grouping, and the total population outcomes. The association of diagnostic grouping and categorized cTn level was assessed using Pearson's chi-square test. All statistical analyses were performed using the programming language R, Version 3.1.2 (http://cran.r-project.org/).

## 3. Results

During the 5-month study period, 23,731 patients were seen in the ED of which 4,928 (21%) had a cTnI testing as part of their ED evaluation (see Figure 1). Among the tested subjects, 804 had an elevated initial cTnI. This represents 3.4% of all patients seen and 16.3% of those that had a cTnI ordered. The leading causes of an elevated cTnI across all diagnostic groups were from congestive heart failure (17%), infection (16%), dysrhythmia (6%), and blood loss (4%). In the low troponin level strata (0.05–0.9 ng/mL) were 383 patients (48%), 339 (42%) fell into the medium strata (0.1–0.99 ng/mL), and 82 (10%) fell into the high strata (≥1.0) (See Table 2). The type of primary diagnosis was significantly associated with categorized cTnI ($P < 0.001$). Eleven percent had a final diagnosis of Type I MI. This was only 1.8% of those who had a troponin level checked and 0.03% of all patients evaluated. Fifteen (17%) had ST-elevation MI and 74 (83%) had non-ST-elevation MI. Eight percent had urgent revascularization.

Among those with Type I MI, 26% had low troponin levels, 36% had medium, and 38% had high. Sixty-six patients (74%) underwent urgent revascularization, and 6% died. These deaths accounted for a minority (7%) of all deaths. Other cardiovascular diagnoses occurred in 277 (35%) of patients with an elevated cTnI. The most frequent diagnoses in this group were congestive heart failure (53%), dysrhythmia (18%), and hypertension (9%). Ten (4%) of these patients died accounting for 12% of all deaths. Noncardiovascular diagnoses were identified for 438 (55%) patients. The top diagnoses in this group were infection (29%), blood loss (7%),

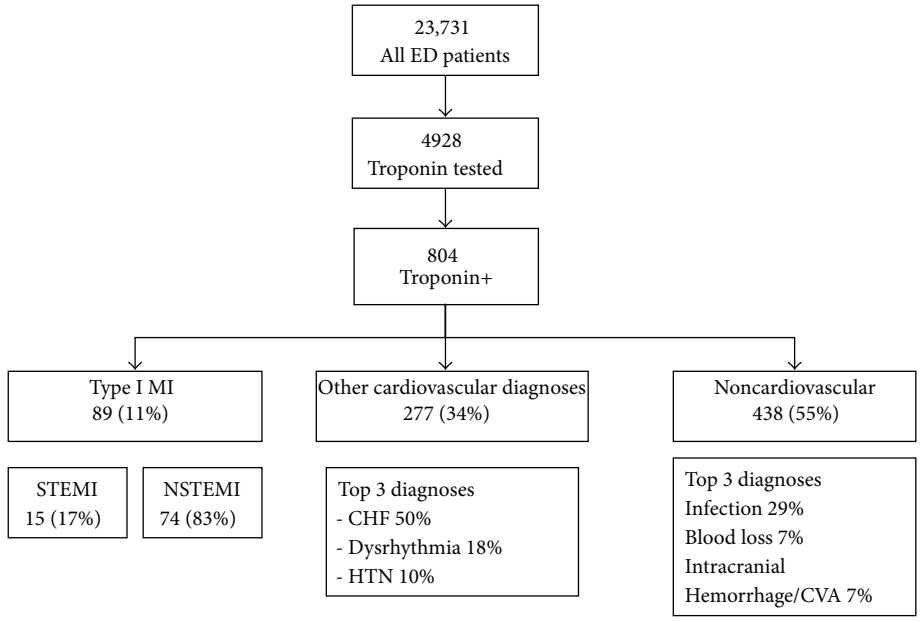


Figure 1: Study population and diagnostic groups.

Table 2: Positive troponin population data analysis by troponin level strata.

| | $N$ | Low $N = 383$ | Medium $N = 339$ | High $N = 82$ | Combined $N = 804$ | $P$ value |
|---|---|---|---|---|---|---|
| Age | 804 | 59 72 82 | 58 70 81 | 57 67 80 | 58 71 81 | 0.22[1] |
| Sex | 804 | | | | | 0.95[2] |
| Female | | 46% (177) | 47% (160) | 48% (39) | 47% (376) | |
| Male | | 54% (206) | 53% (179) | 52% (43) | 53% (428) | |
| First cTnI | 804 | 0.05 0.06 0.08 | 0.12 0.20 0.35 | 1.73 3.00 6.29 | 0.06 0.10 0.27 | <0.001[1] |
| Type of diagnosis | 804 | | | | | <0.001[2] |
| Type 1 MI | | 6% (23) | 9% (32) | 41% (34) | 11% (89) | |
| Other cardiovascular diagnoses | | 39% (148) | 33% (111) | 22% (18) | 34% (277) | |
| Noncardiovascular | | 55% (212) | 58% (196) | 37% (30) | 54% (438) | |
| eGFR | 758 | 30 53 75 | 26 49 72 | 38 64 85 | 29 52 75 | 0.015[1] |
| CKMB | 759 | 1.2 2.4 4.0 | 1.6 3.0 5.3 | 5.1 9.2 21.3 | 1.6 2.9 5.5 | <0.001[1] |
| Creatinine | 758 | 0.90 1.21 2.00 | 0.94 1.30 2.30 | 0.87 1.08 1.60 | 0.90 1.24 2.04 | 0.026[1] |
| MI | 804 | | | | | <0.001[2] |
| No | | 94% (360) | 91% (307) | 59% (48) | 89% (715) | |
| Yes | | 6% (23) | 9% (32) | 41% (34) | 11% (89) | |
| Urgent revascularization | 804 | | | | | <0.001[2] |
| No | | 95% (364) | 94% (320) | 66% (54) | 92% (738) | |
| Yes | | 5% (19) | 6% (19) | 34% (28) | 8% (66) | |
| Status | 804 | | | | | 0.001[2] |
| Alive | | 93% (355) | 88% (299) | 79% (65) | 89% (719) | |
| Died | | 7% (28) | 12% (40) | 21% (17) | 11% (85) | |

*a b c* represent the lower quartile *a*, the median *b*, and the upper quartile for continuous variables.
*N* is the number of nonmissing values.
Numbers after percents are frequencies.
Tests used: [1]Kruskal-Wallis test; [2]Pearson's test.

Table 3: Positive troponin population data analysis by diagnostic groups.

| | $N$ | Type I MI $N = 89$ | Other cardiovascular diagnoses $N = 277$ | Noncardiovascular $N = 438$ | Combined $N = 804$ | $P$ value |
|---|---|---|---|---|---|---|
| Age | 804 | 57 64 77 | 57 69 80 | 60 74 83 | 58 71 81 | <0.001[1] |
| Sex | 804 | | | | | 0.38[2] |
| Female | | 40% (36) | 46% (128) | 48% (212) | 47% (376) | |
| Male | | 60% (53) | 54% (149) | 52% (226) | 53% (428) | |
| First cTnI | 804 | 0.09 0.34 2.49 | 0.06 0.09 0.21 | 0.06 0.10 0.24 | 0.06 0.10 0.27 | <0.001[1] |
| First cTnI (categorized) | 804 | | | | | <0.001[2] |
| Low | | 26% (23) | 53% (148) | 48% (212) | 48% (383) | |
| Medium | | 36% (32) | 40% (111) | 45% (196) | 42% (339) | |
| High | | 38% (34) | 6% (18) | 7% (30) | 10% (82) | |
| eGFR | 758 | 52 73 90 | 30 51 72 | 25 50 72 | 29 52 7 | <0.001[1] |
| CKMB | 759 | 1.9 3.6 9.5 | 1.6 2.7 4.2 | 1.5 2.9 5.6 | 1.6 2.9 5.5 | 0.003[1] |
| Creatinine | 758 | 0.80 1.00 1.26 | 0.90 1.30 2.00 | 0.98 1.30 2.35 | 0.90 1.24 2.04 | <0.001[1] |
| MI | 804 | | | | | <0.001[2] |
| No | | 0% (0) | 100% (277) | 100% (438) | 89% (715) | |
| Yes | | 100% (89) | 0% (0) | 0% (0) | 11% (89) | |
| Urgent revascularization | 804 | | | | | <0.001[2] |
| No | | 26% (23) | 100% (277) | 100% (438) | 92% (738) | |
| Yes | | 74% (66) | 0% (0) | 0% (0) | 8% (66) | |
| Status | 804 | | | | | <0.001[2] |
| Alive | | 94% (84) | 96% (267) | 84% (368) | 89% (719) | |
| Died | | 6% (5) | 4% (10) | 16% (70) | 11% (85) | |

*a b c* represent the lower quartile *a*, the median *b*, and the upper quartile for continuous variables.
*N* is the number of nonmissing values.
Numbers after percents are frequencies.
Tests used: [1]Kruskal-Wallis test; [2]Pearson's test.

and intracranial hemorrhage or stroke (7%). Seventy (16%) of these patients died and accounted for 82% of all deaths.

In examining the troponin level (see Table 3) we found that within the low strata 6% had a final diagnosis of Type I MI, 5% underwent urgent revascularization, 39% had other cardiovascular diagnoses, 55% of patients had a noncardiovascular diagnosis (see Figure 2), and 7% died. These deaths were 33% of all deaths. Within the medium strata, 9% had an end diagnosis of Type I MI, 6% of the group experienced urgent revascularization, 33% had other cardiovascular diagnoses, 58% had a noncardiovascular diagnosis, and 12% died. These deaths accounted for 47% of all deaths. The leading diagnoses within this group were dysrhythmia with atrial fibrillation accounting for all cases. In the high strata the leading diagnoses were Type I MI, AICD firing, and blood loss. Within this group 41% of patients had Type I MI, 34% had urgent revascularization, 22% had other cardiovascular diagnoses, 37% had a noncardiovascular diagnosis, and 21% of all patients with a high troponin died. This was 20% of all deaths.

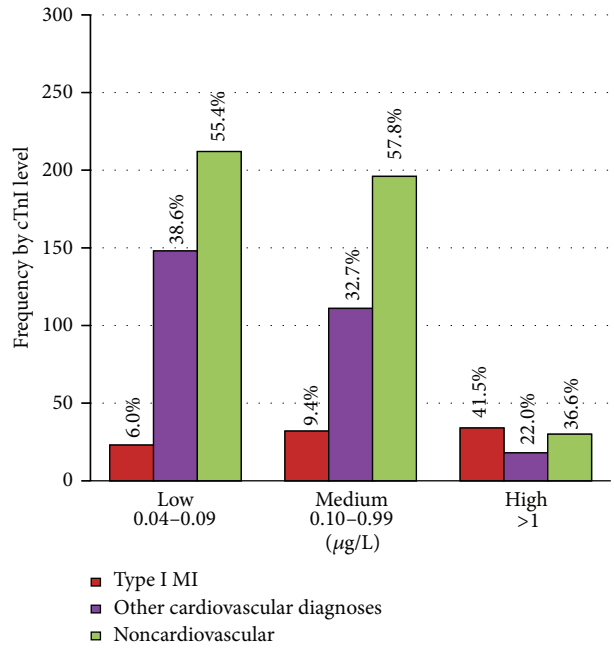


Figure 2

## 4. Discussion

This study demonstrates that myocardial infarction is the cause of troponin elevation in a minority of cases. The striking finding from the study is the diversity (see Figure 1) within the differential diagnosis for an elevated cTnI in an undifferentiated patient population. The majority of deaths among those with a positive troponin (82%) were attributed

to noncardiovascular diagnoses. Our results support prior work that has identified the incidence of MI in this population to be between 9 and 13% and the troponin elevations to be mostly attributed to noncardiac diagnoses [10, 11]. However, we also found that patients with high initial troponin levels had a much higher incidence of Type I MI (see Figure 2). The result of the first troponin test in the ED helps gauge the likelihood of admission versus discharge and the likely focus of early in-hospital care. Unlike other MI types, treatment is focused on early antithrombotic coronary artery therapies. Thus the likelihood of a positive initial troponin being Type I MI is of high importance in priming these time-sensitive interventions.

The specificity of a cTn elevation being attributed to Type I MI increases with serial testing. Because of this, the 2014 American College of Cardiology/American Heart Association guidelines for the diagnosis and management of NSTEMI advise measuring troponin levels at 3–6 hours after symptoms onset and beyond 6 hours for patients with a moderate to high risk [12]. However, an ideal ED evaluation is completed at 4–6 hours, and the report of symptom onset is not always reliable upon early patient evaluation in the ED. This prompts many physicians to use ED arrival as their start time for serial testing. As a result, decisions on whether to admit or discharge patients, particularly when the initial troponin is positive, are often made before serial testing is completed.

In addition, many ED patients are undifferentiated, thus undergoing simultaneous evaluations for other cardiovascular and noncardiovascular conditions that extend into their in-hospital stay. A troponin elevation is only a marker of myocardial injury, which can result from multiple mechanisms that cause myocyte death [13] including ischemia from acute coronary syndrome (which includes STEMI and NSTEMI), surgical trauma [14], mechanical trauma [15], poorly understood neurohormonal and inflammatory processes, and systemic demand [16]. Insight into the likely differential diagnosis of an elevated troponin can help receiving in-hospital teams identify and target the true cause of myocardia injury with more clarity.

The findings of this study shed light on the likely differential diagnosis when myocardial injury is demonstrated by an increased troponin level. Specifically, we found that just under half of all positive tests were attributed to CHF, infection, dysrhythmia, or blood loss. The quest for increased sensitivity in identifying potential MI in patients has led to biomarkers that have dramatically increased the number of patients with a positive test. This study was performed at time when this institution was switching from a cTnI assay to the more sensitive cTnT. Both the department of pathology and emergency medicine wanted to better understand the end diagnostic nature of false positive tests when a troponin was used to screen for myocardial infarction as a subset of myocardial injury and to better understand the alternative diagnoses to consider with a positive test.

Regardless of the etiology, an elevated cTnI result often prompts a hospital admission for further investigation [17]. Serial troponin testing is more sensitive for the ultimate diagnosis of Type I MI [18]. However, two 2009 studies by Gudmundsson et al. [3] and Amsterdam et al. [12] note the diagnostic sensitivity of an initial ultrasensitive or high-sensitive ED cTnI assay in the early diagnosis of Type I MI. Biochemical evidence of myocardial injury in the context of concerns for MI will prompt an in-hospital stay, because this finding increases the risk of a negative outcome regardless of the etiology [3]. In our study we observed that mortality trended with the level of troponin elevation.

## 5. Study Limitations

Some limitations of our study should be considered. This is a retrospective study including data collected from a heterogeneous mix of patients. Each patient had a cTnI blood level sent by a heterogeneous group of emergency medicine physicians that were not using a standard protocol to screen for myocardial injury or infarction. There is no strict policy or protocol for when to test for a troponin I level in this institution. However, all troponins require a physician order to be processed. We did not follow the final diagnosis of patients whose cTnIs were negative during their ED stay or any associated hospital stay. This prevented us from identifying the negative and positive predictive values of the initial ED troponin in the population of patients being evaluated for Type I MI and we did not assess the implications of serial troponin testing as this was outside the scope of this study. Many "other cardiovascular" diagnoses qualify as MI. However, they are typically Type II MI which is from increased oxygen demand or decreased oxygen supply to the demand or restrictions of another cardiovascular pathology. However, given our intent to identify those patient who will receive early acute coronary syndrome intervention this study is limited to the identification of individuals with Type I MI.

## 6. Conclusion

Concerns for potential MI may be the primary reason for testing, but a minority of patents with a positive result had Type I MI as their final diagnosis. Most common diagnoses were congestive heart failure, infections, dysrhythmias, and blood loss. In addition, the majority of deaths were due to alternative diagnoses with most falling in the noncardiovascular diagnostic group. This supports existing evidence that myocardial injury is a marker of increased morbidity and mortality. We also observed an increasing trend in mortality correlating to the level of troponin elevation. In comparing troponin strata, Type I MI was more common in the medium strata than the low strata and significantly more present in the high troponin strata. Overall, a patient who has a positive cTnI will most likely have other cardiovascular or noncardiovascular diagnoses since only 11% of all patients had Type I MI.

## Conflict of Interests

All the authors but Dr. Yiadom certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants;

participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interests; and expert testimony or patent-licensing arrangements), or nonfinancial interest (such as personal or professional relationships, affiliations, and knowledge or beliefs) in the subject matter or materials discussed in this paper.

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*Review Article*

# Copeptin Testing in Acute Myocardial Infarction: Ready for Routine Use?

**Sebastian Johannes Reinstadler, Gert Klug, Hans-Josef Feistritzer, Bernhard Metzler, and Johannes Mair**

*Department of Internal Medicine III, Cardiology and Angiology, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria*

Correspondence should be addressed to Johannes Mair; johannes.mair@i-med.ac.at

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Suspected acute myocardial infarction is one of the leading causes of admission to emergency departments. In the last decade, biomarkers revolutionized the management of patients with suspected acute coronary syndromes. Besides their pivotal assistance in timely diagnosis, biomarkers provide additional information for risk stratification. Cardiac troponins I and T are the most sensitive and specific markers of acute myocardial injury. Nonetheless, in order to overcome the remaining limitations of these markers, novel candidate biomarkers sensitive to early stage of disease are being extensively investigated. Among them, copeptin, a stable peptide derived from the precursor of vasopressin, emerged as a promising biomarker for the evaluation of suspected acute myocardial infarction. In this review, we summarize the currently available evidence for the usefulness of copeptin in the diagnosis and risk stratification of patients with suspected acute myocardial infarction in comparison with routine biomarkers.

## 1. Introduction

The discovery of the biomarker cardiac troponin (cTn) as well as its introduction as a test into clinical routine has been one of the most important advances in the evaluation of patients with suspected acute myocardial infarction (AMI) over the last decades. Today, cTn plays a key role in the management of patients with acute coronary syndromes (ACS) [1]. A further clinically relevant increase in the sensitivity of cTn at an early diagnostic stage was achieved with the introduction of high-sensitivity (hs) cTn assays [2–4]. Despite these advances, there remains a troponin-blind period very early after symptom onset. Therefore, in patients with suspected AMI, a rule-out process with cTn measurement at presentation and 3 hours thereafter is still required when hs-cTn assays are used [5, 6]. Efforts to discover new biomarkers enabling a reliable earlier rule-out of AMI and thus a reduction of unnecessary hospital admissions are continuing. Besides its pivotal role as a diagnostic tool, cTn provides also information for risk assessment in the setting of ACS and many other cardiac and noncardiac diseases [5, 7–14]. Other promising biomarkers for risk stratification are natriuretic peptides (NPs) [7, 15, 16] and high-sensitivity C-reactive protein (hs-CRP) [17–19]. In the context of AMI, however, the incremental value of these biomarkers beyond conventional risk factors seems to be only moderate, and true large-scale comparative studies are still missing. Therefore, the role of novel biomarkers other than that of the routinely used cTn, NPs, and hs-CRP that might enable a better risk stratification of patients with chest pain is being increasingly investigated [20–25]. One of the most frequently proposed and extensively investigated biomarkers for facilitating the diagnosis of AMI is copeptin [6]. In addition, copeptin was also evaluated for risk stratification in this patient cohort. In this review, we will summarize the current clinical evidence for its routine use in patients with suspected AMI.

## 2. Pathophysiology of Copeptin

Located on chromosome 20, the gene named arginine vasopressin (AVP) encodes a 164-amino-acid peptide called pre-

pro-AVP, which is produced by neurons of the hypothalamo-neurohypophysial system [26]. The mature pre-pro-AVP is the precursor molecule for AVP, which also includes a signal peptide, neurophysin II, and copeptin [27]. Copeptin (or C-terminal provasopressin) is a glycosylated 39-amino-acid peptide. As physiological function copeptin is believed to be involved in the proper folding of pre-pro-AVP [28, 29]. After transportation from the hypothalamus to the pituitary gland and cleavage of the pre-pro-AVP, copeptin is released into the circulation in stoichiometric amounts along with AVP. Both neuropeptides are primarily cosecreted in response to hemodynamic or osmotic alterations. The measurement of circulating AVP is challenging since AVP is an unstable molecule and because it is mainly bound to platelets [30–32]. Unlike AVP, copeptin is relatively stable in the circulation and methodologically easier to determine [31]. Therefore, copeptin is used as a surrogate marker for AVP release and an assay suitable for routine use has been developed [31].

In recent years, copeptin has been considered as a promising biomarker in numerous acute illnesses [33]. For instance, an association between elevated levels of copeptin and an unfavourable outcome were reported in patients with lower respiratory tract infections [34], sepsis [35], stroke [36], and acute pancreatitis [37]. These studies have consistently demonstrated a positive association between copeptin and disease severity. The role of copeptin has also gained particular attention in patients with AMI. Circulating copeptin levels are significantly higher during the acute phase in patients with AMI compared with healthy control subjects [38]. The copeptin elevation is again greater in patients with ST-segment elevation myocardial infarction (STEMI) than in patients with non-ST-segment elevation acute coronary syndrome (NSTE-ACS). The main trigger for copeptin release after AMI is thought to be acute endogenous stress [39, 40]. On the other hand, copeptin secretion is also associated with changes in fluid status [41]. Thus, it can be assumed that hemodynamic changes occurring in the acute phase during AMI might also trigger copeptin release [40]. In an animal study by Hupf et al., it was shown that vasopressin is also expressed in cardiac tissue [42]. Some authors speculate that myocardial necrosis could therefore directly lead to copeptin release from the heart [40, 43]. The release pattern of copeptin was recently described in detail [40, 43]. It is important to note that, in contrast to the MB isoenzyme of creatine kinase (CK-MB) and cTn, copeptin concentrations rise immediately after symptom onset and decrease rapidly thereafter (Figure 1). A direct association between the amount of released copeptin, on the one hand, and acute as well as chronic infarct size determined by cardiac magnetic resonance imaging, on the other, was demonstrated in STEMI patients [44, 45]. It is important to note, however, that, in contrast to cTn, increased copeptin levels are not specific for myocardial damage (see Table 1). For instance, Stallone et al. showed that increased levels of copeptin were measureable in about one-fifth of patients presenting to the emergency department with noncardiac chest pain [46].

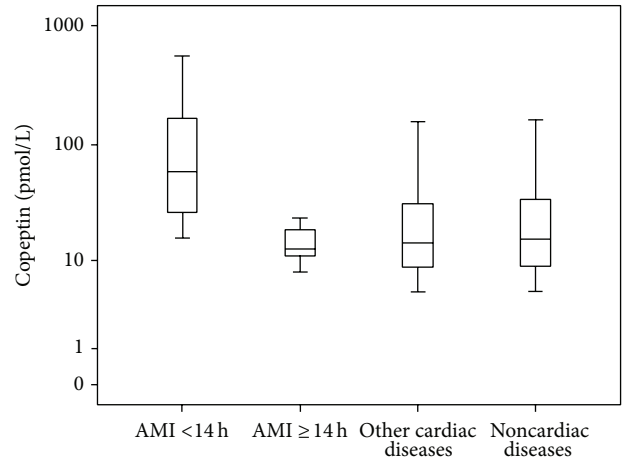


Figure 1: Distribution pattern of copeptin in patients with chest pain admitted to the emergency department ($n = 171$) according to discharge diagnosis. Our own unpublished data are shown as box plots. AMI patients were divided by delay from symptom onset. Copeptin concentrations in AMI patients presenting within 14 h from symptom onset were significantly ($P = 0.013$) higher than in the remaining patients, whereas AMI patients presenting thereafter did not differ significantly. Abbreviations—AMI: acute myocardial infarction.

## 3. Copeptin for the Diagnosis of Acute Myocardial Infarction

*3.1. "Rule-In" of Acute Myocardial Infarction.* As mentioned above, copeptin shows only low specificity for myocardial damage. Accordingly, the positive predictive value (PPV) for AMI of copeptin alone is thought to be unacceptably low. Indeed, the first studies investigating the diagnostic value of copeptin for AMI showed a very low PPV for AMI [47, 48]. For instance, Reichlin et al. calculated the PPV of copeptin for AMI diagnosis for different cut-off concentrations (9 pmol/L, 14 pmol/L, 20 pmol/L, and 24 pmol/L). In their study, the PPV of copeptin ranged between 34.9% and 57.9%. Two recently published meta-analyses confirmed that copeptin alone provides only insignificant diagnostic value in the setting of suspected AMI [49, 50]. Therefore, copeptin alone should not be considered as a single diagnostic marker in patients with suspected ACS.

*3.2. "Rule-Out" of Acute Myocardial Infarction of Copeptin in Combination with Standard cTn and hs-cTn.* On the basis of its unique release pattern, it was speculated that the combination of copeptin with cTn might facilitate the early "rule-out" of AMI. In fact, in the landmark trial published by Reichlin et al. in 2009, the authors concluded that the combination of copeptin and cTn enables a rapid and safe rule-out of AMI at presentation [47]. In their study, they investigated 487 unselected emergency department (ED) patients with symptoms suggestive of AMI. The combination of copeptin and cTn reached a sensitivity of 98.8% and a negative predictive value of 99.7% for ruling-out of AMI already at presentation. The combination performed significantly better

Table 1: Overview of clinical conditions other than AMI associated with increased copeptin concentrations.

| Condition | Potential implications of elevated copeptin concentrations | References |
|---|---|---|
| Stable coronary artery disease | Predictor for major adverse cardiovascular events | [51] |
| Heart failure | Associated with mortality risk, risk of hospitalization, and disease severity | [52–56] |
| Type 2 diabetes | Potential marker for peripheral arterial disease and diabetic chronic kidney disease. Potential marker for cardiovascular and all-cause mortality | [57–59] |
| Pneumonia | Marker for adverse outcome | [60, 61] |
| Acute exacerbation of chronic obstructive pulmonary disease | Potential prognostic marker for short-term and long-term outcome | [62] |
| Sepsis/shock | Promising independent prognostic markers for mortality | [33, 35, 63] |
| Survivors of cardiac arrest | Potentially useful for risk stratification at the time of hospital admission | [64] |
| Pulmonary arterial hypertension | Potentially useful in the prediction of poor outcome | [65] |
| Stroke/transient ischaemic attack | Risk stratification for patients with transient ischaemic attack and stroke | [66–68] |
| Traumatic brain injury | Probable marker of progressive haemorrhagic injury, acute traumatic coagulopathy, and mortality | [69–71] |
| Intracerebral haemorrhage | Useful to predict adverse clinical outcomes | [72, 73] |
| Carotid endarterectomy | Probable predictor of perioperative stroke | [74] |
| CABG surgery | Postoperative copeptin concentrations might predict delirium and cognitive dysfunction | [75] |
| Chronic kidney disease | Potential marker for the development/progression of atherosclerosis | [76] |
| Autosomal dominant polycystic kidney disease | Potential role in disease progression | [77, 78] |
| Carbon monoxide poisoning | Associated with intoxication severity and potentially useful to predict delayed neurological sequelae | [79] |
| Polycystic ovary syndrome | Relationship with cardiometabolic parameters (e.g., carotid intima media thickness) | [80] |
| Endometriosis | Direct association with disease severity | [81] |
| Preeclampsia | Associated with increased risk for preeclampsia already before clinical diagnosis | [82, 83] |
| Acute pancreatitis | Marker for disease severity and local complications | [37, 84] |
| Liver cirrhosis | Associated with the severity of disease and with the risk of death or liver transplantation | [85] |
| Sickle cell anaemia | Differentiation between mild or severe sickle cell anaemia | [86] |

Aortocoronary bypass grafting (CABG).

compared to cTn alone. In addition, with the use of the dual marker strategy, the diagnostic accuracy was high for the diagnosis of AMI at presentation (area under the curve: 0.97). Nevertheless, some important limitations have to be mentioned. Although the cohort was comparable with other similar trials, it has all the limitations of a single-centre study. More importantly, a non-hs cTn assay was used, which was appropriate at that time but would not be today. Following this study, there have been a great number of reports confirming [48, 87–94] or rejecting [23, 95–99] this hypothesis. There are various reasons for these conflicting results. As mentioned before, one crucial point is the use of non-hs-cTn assay. hs-cTnT assays have been shown to provide a better early diagnostic sensitivity for AMI in the ED compared with the previous cTn assay generations [2, 100, 101]. Not surprisingly, almost all studies comparing copeptin with cTn assessed by a conventional assay display a substantial benefit of the dual marker approach. In contrast, when an hs-cTn is used, the benefit seems to be only moderate or absent [49] (Figure 2). The additive value seems to be especially low when the limit of detection (LoD) of hs-cTn is used as a decision limit for ruling out of AMI. This is of little surprise, since trials using LoD as "rule-out" criterion found a negative predictive value of up to 100% [102, 103]. On the other hand, a more sensitive assay for the determination of copeptin has been developed and is available for routine use as well [91]. This assay enables a more precise measurement of copeptin, which could also explain in part the differences in the "rule-out" studies. Moreover, initially performed studies used a copeptin cut-off value of 14 pmol/L, whereas recent data suggest that 10 pmol/L might be a more appropriate decision limit [49]. Another vital issue is the time point of copeptin sampling. As mentioned earlier, copeptin concentration increases to maximum immediately after symptom onset and decreases within hours thereafter. Hence, the proposed dual marker strategy is only reasonable in early presenters and when blood

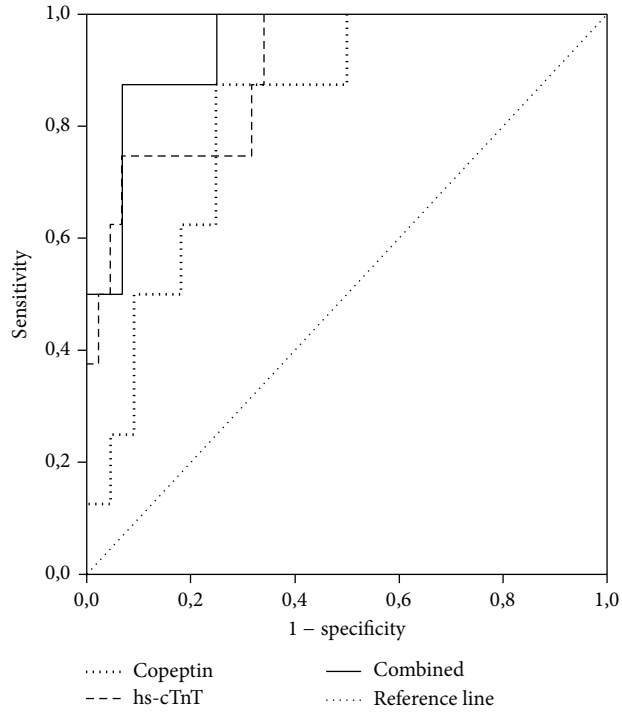


Figure 2: ROC analysis to compare the diagnostic power of copeptin, hs-cTnT, and the combination of both for the diagnosis of AMI in patients presenting with chest pain early after symptom onset (within 14 hours) on admission. Own unpublished data. The AUC of hs-cTnT (0.90, 95% confidence interval 0.79–0.97) did not differ significantly from the AUC of copeptin combined with hs-cTnT (0.94, 95% confidence interval 0.84–0.99; $P > 0.05$). Abbreviations—ROC: receiver operating characteristic; hs-cTnT: high-sensitivity cardiac troponin T; AMI: acute myocardial infarction; AUC: area under the curve.

samples are drawn as early as possible. This fact might explain some negative studies when copeptin was measured with a notably delay after patient presentation [98].

*3.3. "Rule-Out" of Acute Myocardial Infarction—Meta-Analysis and Interventional Trials.* Meta-analyses performed so far have concluded that copeptin added to cTn results in significantly increased sensitivity for the diagnosis of AMI compared with cTn alone [49]. Of note, they also conclude that copeptin is most useful when combined with conventional cTn, and the added value remains uncertain when hs-cTn is used. The results of this meta-analysis, however, have to be interpreted with caution. Considerable heterogeneity among studies limits the interpretation of data. Furthermore, the literature search for this meta-anylisis was carried out in 2013. Important negative studies published in 2014 [95, 96] are therefore not included in analysis.

Probably the most important drawback of all above-mentioned studies is their observational design. In these studies, the measured copeptin value did not influence patient management. Management studies, however, showing that this novel approach is effective and safe are indispensable before copeptin can be recommended for routine use. Recently, the first and so far the only randomized, multicentre clinical process trial was published [104]. In this very important study by Möckel et al., 902 low-risk patients with suspected ACS were randomly assigned to either standard care (serial cTn testing as recommended) or an experimental arm. In the experimental arm, copeptin was measured in addition to cTn and if both biomarkers tested negative, the patient was eligible for discharge without the need for serial cTn testing. It needs to be mentioned that, in the latter case, the treating physician could still decide not to discharge the patient immediately. The primary safety study end-point was defined as combination of all-cause death, survived sudden cardiac death, AMI, rehospitalization for ACS, acute unplanned percutaneous coronary intervention, coronary artery bypass grafting, and documented life-threatening arrhythmia 30 days after enrollment. The authors found a similar event rate (standard arm: 5.17%, experimental arm: 5.19%) in both study arms, suggesting that the combined cTn/copeptin strategy is as safe as the standard procedure. The advantage of the new approach was also clearly demonstrated. In the experimental arm, hospital stay was significantly shorter compared with standard arm (4 versus 7 hours, resp., $P < 0.001$). The main conclusion was therefore that a dual marker strategy with copeptin and cTn could safely decrease length of hospital stay. Although the study was well designed, there are some relevant limitations that have to be considered. cTn was measured by different conventional (three sites) as well as high-sensitive assays (four sites) across the study sites. Furthermore, the thresholds for a "negative" cTn result were defined by the 99th percentile upper reference limit. By using the LoD as cut-off, the negative predictive value of cTn can be further increased as demonstrated by recent studies [103]. In the era of hs-cTn assays, a crucial study would be the direct of comparison of hs-cTn versus copeptin plus hs-cTn with the LoD as a rule-out cut-off for hs-cTn. Whether copeptin still provides an incremental value in this case remains to be investigated.

*3.4. "Rule-Out" of Acute Myocardial Infarction in Point-of-Care Testing.* In times of overcrowding of the emergency departments, point-of-care testing (POCT) becomes increasingly appealing. Recent evidence suggests that POCT might allow a fast and accurate diagnosis of AMI [105]. Nowadays, cTn is the most efficient diagnostic marker also in POCT [105]. Because of the lower sensitivity of POCT, cTn assays [106] compared to assays used in the central laboratory and the early period of "troponin blindness" novel biomarkers might improve the early diagnostic sensitivity in POCT. The use of the CK-MB and myoglobin was recently tested but failed to improve diagnostic performance [105]. The different release kinetics of copeptin compared with cardiac troponin after AMI makes copeptin a very promising candidate for POCT [40]. Furthermore, the encouraging results for the combination of copeptin with standard troponin assays used in central laboratory analysis suggest that a significant improvement might be possible also in POCT. Till now, prospective randomised trials are missing, but a dual marker POCT strategy including copeptin and cTn is worth being prospectively evaluated in future studies.

## 4. Copeptin for Risk Stratification in Acute Coronary Syndromes

In order to optimize timing and intensity of therapeutic interventions as part of patient management, adequate risk stratification at an early stage after ACS is necessary [5]. Early echocardiography data revealed that copeptin concentrations, assessed 3–5 days after AMI, are correlated with left ventricular dysfunction as well as remodelling 5 months after the event [107]. More recent cardiovascular magnetic resonance studies confirmed the association between copeptin, myocardial function, and adverse remodelling following STEMI treated with primary coronary intervention [44, 45]. Interestingly, the combination of day 2 copeptin and NT-proBNP levels could exclude the development of adverse remodelling over 4 months after AMI. One might therefore speculate that STEMI patients with increased copeptin concentrations after revascularization might benefit from more intense therapeutic regimens [108].

Whether copeptin is of prognostic value among patients with AMI was studied for the first time by Khan et al. in the Leicester Acute Myocardial Infarction Peptide (LAMP) study [38]. In this single-centre study of 980 consecutive patients with AMI, increased values of copeptin (odds ratio: 4.14, $P < 0.0005$) measured between days 3 and 5 after AMI were associated with the primary end-point of death or heart failure at 60 days in multivariate analysis. The area under the curve (AUC) for the prediction of the primary end-point for copeptin (0.75) was similar to that of NT-proBNP (0.76). Interestingly, the combination of both biomarkers led to a significant improvement of AUC (0.84), suggesting that a dualmarker strategy might be more useful for risk assessment in patients suffering an AMI. Because approximately 80% of the included patients in LAMP had a STEMI, a second study with only non-ST segment elevation-ACS (NSTE-ACS) patients was performed by the same study group [109]. The LAMP II study showed in 754 NSTE-ACS patients that copeptin (measured within 24 hours after admission) is an independent predictor of all-cause mortality at 6 months. In contrast to NT-proBNP, copeptin yielded a significant net reclassification improvement of 13% ($P = 0.008$) when added to the GRACE score.

The prognostic utility of copeptin levels on admission to hospital in patients with suspected AMI was recently documented in a prospective, single-centre study by Afzali et al. [110]. In this study of 230 patients, 107 had the final diagnosis of AMI (24 STEMI and 83 NSTE-ACS). The authors showed that levels of copeptin on admission significantly predict 180-day mortality. The AUC of copeptin (0.81) was higher compared with the AUC of cTnI (0.76) and the combination of both biomarkers (0.83) performed again better than either marker alone. Although copeptin measured at admission in 377 NSTE-ACS patients was related to death within one month after the index event, this association did not remain significant after adjusting for baseline characteristics or cTn levels in the COPED-PAO study [111].

To further elucidate the value of biomarkers in the post-AMI risk assessment, a recently published large study by O'Malley et al. compared the prognostic performance of multiple biomarkers sampled at enrollment among 4,432 prospectively recruited subjects with NSTE-ACS [20]. The authors conclude that, although cTn-I performed best among all investigated outcomes, copeptin seems a robust prognosticator for cardiovascular death and heart failure beyond established biomarkers. Therefore, copeptin appears promising for improving risk stratification in conjunction with other biomarkers. Of note, the authors could confirm previous data from the LAMP study indicating that copeptin is less suited to predict recurrent ischaemia. This might be explained by the fact that copeptin is primarily released in response to hemodynamic stimuli, but not by progression of atherosclerosis. A meta-analysis published in 2014 showed that the predictive value of copeptin and cTn for all-cause mortality is the same [49].

A relevant limitation of the above-mentioned studies is that none of these compared copeptin with hs-cTn. One study investigating the combination of these two biomarkers was recently published [112]. Patients with preexisting coronary artery disease and symptoms indicating AMI ($n = 433$) were analysed in a prospective multicentre fashion. Copeptin determined on admission provided prognostic information for the risk of death at 1 year after enrollment. More importantly, the combination of copeptin with hs-cTn yielded significantly enhanced prognostic accuracy. Further investigations are warranted to confirm these promising data.

A further important question is the time point of copeptin testing. Great between-study heterogeneity exists regarding the time point of copeptin sampling. Studies measuring copeptin at different time points during the (sub-) acute phase after ACS are lacking and therefore the optimal time point for assessing copeptin concentration remains unknown.

Another major drawback of the currently available evidence is that no study evaluated a copeptin (or multimarker) based therapeutic decision pathway. Prospective interventional trials are warranted to elucidate if measurement of copeptin provides additional information beyond established risk tools that impact treatment decisions which might improve patient outcome.

## 5. Summary

For the diagnostic evaluation of AMI, cTn remains the "gold standard" biomarker. There is enough evidence from observational studies indicating that a dual marker strategy combining measurments of copeptin and cTn levels using a conventional assay might facilitate the "rule-out" of AMI in early presenters. Also in POCT, such a dual strategy seems promising, but randomized clinical trials are lacking. However, when hs-cTn assays are used, the advantage of this approach seems insignificant. Data from a first randomized, controlled clinical process trial are promissing as they suggest that this new strategy allows early and safe discharge, but further prospective interventional trials are needed to confirm those results for the combination of copeptin with hs-cTn. Real-world data from large regrities are also necessary to accurately evaluate this strategy. Therefore, based on the currently available body of evidence, we do not believe that

copeptin testing can yet be recommended for use in routine clinical practice if hs-cTn assay is used.

For prognostic evaluation, current data support the use of copeptin, integrated into a multimarker approach, to improve the classification of AMI patients into different risk groups early after the acute event. However, studies showing that a biomarker-guided strategy for risk stratification improves patient outcome are needed before testing for copeptin (and other biomarkers) can be recommended for implementation in clinical routine.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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*Research Article*

# Evaluation of High Sensitive Troponin in Erectile Dysfunction

**Alessandra Barassi,[1] Raffaele Pezzilli,[2] Antonio Maria Morselli-Labate,[2] Elena Dozio,[3] Luca Massaccesi,[4] Francesca Ghilardi,[1] Clara Anna Linda Damele,[1] Giovanni Maria Colpi,[5] Gian Vico Melzi d'Eril,[1] and Massimiliano Marco Corsi Romanelli[3,6]**

[1] *Laboratorio di Analisi, Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, 20142 Milano, Italy*
[2] *Dipartimento di Malattie dell'Apparato Digerente e Medicina Interna, Ospedale Sant'Orsola-Malpighi, Alma Mater Studiorum, Università degli Studi di Bologna, 40138 Bologna, Italy*
[3] *Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, 20133 Milano, Italy*
[4] *Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università di Milano, 20133 Milano, Italy*
[5] *Istituto per la Sterilità e la Sessualità (ISES), 20123 Milano, Italy*
[6] *Unità Operativa Medicina di Laboratorio-1 Patologia Clinica, IRCCS Policlinico San Donato, San Donato Milanese, 20097 Milano, Italy*

Correspondence should be addressed to Alessandra Barassi; alessandra.barassi@unimi.it

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*Background.* Evidence is accumulating in favour of a link between erectile dysfunction (ED) and coronary artery diseases. We investigated the presence of cardiac injury in patients who have had arteriogenic and nonarteriogenic ED using the hs-Tn levels. *Methods.* The diagnosis of ED was based on the International Index of Erectile Function 5-questionnaire (IIF-5) and patients were classified as arteriogenic (A-ED, $n = 40$), nonarteriogenic (NA-ED, $n = 48$), and borderline (BL-ED, $n = 32$) patients in relation to the results of echo-color-Doppler examination of cavernous arteries. The level of hs-TnT and hs-TnI was measured in 120 men with a history of ED of less than one year with no clinical evidence of cardiac ischemic disease. *Results.* The levels of both hs-TnT and hs-TnI were within the reference range and there was no significant ($P > 0.05$) difference between patients of the three groups. The hs-CRP values were higher in A-ED men compared with NA-ED ($P = 0.048$) but not compared with BL-ED ($P = 0.136$) and negatively correlated with IIF-5 ($r = -0.480$; $P = 0.031$). *Conclusions.* In ED patients of the three groups the measurement of hs-Tn allows us to exclude the presence of cardiac involvement at least when the history of ED is less than one year and the men are without atherosclerotic risk factors.

## 1. Introduction

Erectile dysfunction (ED) is now considered an early manifestation of a largely subclinical systemic vascular disorder affecting also the penile arteries. Indeed, ED shares with other vascular diseases, mainly coronary artery disease (CAD), common risk factors [1–4] and a similar pathogenic involvement of the NO pathway that leads to early impairment of endothelium-dependent vasodilatation and late obstructive vascular changes [5–8].

Cardiac troponin T (TnT) and troponin I (TnI) are the markers recommended for diagnosis of acute myocardial infarction [9]. With the implementation of assays with improved analytical sensitivity (hs-Tn), the reliable determination of troponin, even at concentrations below the previously defined 99th percentile values, has become possible. The limit of detection for the newest generation of hs-Tn assay is 10- to 100-fold lower than that of traditional assays [10]. Although an increase in troponin concentration classically indicates myocyte necrosis, several cellular processes other than necrosis can induce troponin release. Among them is increased cell wall permeability which may occur because of ischemia [11]. Test-induced ischemia has been associated with a quantifiable increase of troponin detectable with an

ultrasensitive assay in proportion to the grade of ischemia, that is, mild or moderate to severe [12]. In addition to providing diagnostic information, the troponin levels offer powerful prognostic information even if detected in asymptomatic adults and moreover several cardiovascular risk factors have been shown to correlate with hs-Tn levels [13]. The emerging awareness of ED as an early warning for vascular health and possibly of silent CAD represents a unique opportunity to improve preventive cardiovascular health in all adult men who have persistent difficulty in achieving or maintaining an erection [14]. However, it is vital for effective management of the condition to isolate from this group of men those at high risk of CAD in particular to avoid treating those individuals who are not at risk of CAD and therefore who do not need it. The aim of our investigation was the early detection of cardiac injuries, even where no symptoms were present, using hs-Tn assays, in patients who have had ED symptoms for a few months, more than three months but less than one year, without known CAD risk factors. In fact, these patients are particularly susceptible to the development of CAD and would benefit the most from lifestyle modification or appropriate medical management earlier.

## 2. Material and Methods

Out of a series of 590 filed ED cases studied in the period of October 2009 to June 2011, we included in this study all patients with a history of ED of more than 3 months but less than one year. Exclusion criteria were clinical evidence from the patient's clinical history of coronary artery disease, dyslipidemia, arterial hypertension, malignancies, renal failure, congestive heart failure, systemic inflammatory disease, rheumatic disease, infection diseases, anemia, chronic liver diseases, arrhythmias, trauma, and no current smoking and vitamins supplementation or chronic drug assumption [15–19]. This investigation conforms to the principles outlined in the Declaration of Helsinki. Signed informed written consent was obtained from all subjects before their participation in the study. No ethical committee approval was required because no additional blood was needed for this study, and this was explained thoroughly to all patients, and also because this procedure has been reported to be acceptable [20–23].

On the basis of the given inclusion and exclusion criteria reported above, we selected a total of 120 ED patients who were included in the study. Their clinical characteristics are summarized in Table 1. The ED was classified as arteriogenic origin in 40 patients and as nonarteriogenic origin in 48 patients and, finally, in 32 patients the ED was classified as borderline. In our center, patients complaining of ED are currently investigated by careful history-taking and clinical andrological examination, then, a few days later, by a panel of examinations, including blood tests such as hemoglobin, glycated hemoglobin, glycemia, creatinine, high-sensitivity C-reactive protein (hs-CRP), total and HDL cholesterol, triglycerides, transaminases, testosterone, prolactin, 17-$\beta$-estradiol, urinalysis and 24 h urinary albumin excretion (microalbuminuria), the International Index of Erectile Function questionnaire (IIEF), and echo-color-Doppler of both cavernous arteries.

Table 1: Clinical and laboratory features of patients with erectile dysfunction. Values are reported as mean and standard deviation.

| | NA-ED ($n = 40$) | A-ED ($n = 48$) | BL-ED ($n = 32$) |
|---|---|---|---|
| Patients data | | | |
| Age (years) | 52.0 ± 8.5 | 58.1 ± 7.3 | 50.0 ± 10.5 |
| IIEF-5 score | 12.8 ± 2.4 | 10.5 ± 2.6 | 11.6 ± 1.8 |
| Laboratory evaluation | | | |
| Study parameters | | | |
| hs-cTnT (pg/mL) | 2.89 ± 5.01 | 4.32 ± 7.29 | 3.10 ± 4.18 |
| hs-cTnI (pg/mL) | 4.72 ± 10.93 | 2.4 ± 6.25 | 0.79 ± 3.88 |
| hs-CRP (mg/L) | 1.70 ± 1.78 | 3.09 ± 3.19* | 2.03 ± 2.79 |
| Other parameters | | | |
| Creatinine (mg/dL) | 0.92 ± 0.16 | 0.95 ± 0.16 | 0.96 ± 0.15 |
| Glucose (mg/dL) | 102.9 ± 6.3 | 93.0 ± 14.1 | 95.6 ± 10.8 |
| ALT (U/L) | 26.22 ± 15.76 | 23.10 ± 10.29 | 24.46 ± 8.29 |
| AST (U/L) | 23.56 ± 10.16 | 21.60 ± 5.71 | 27.29 ± 10.28 |
| Total cholesterol (mg/dL) | 200.36 ± 10.21 | 207.80 ± 11.72 | 205.21 ± 9.49 |
| HDL cholesterol (mg/dL) | 55.14 ± 13.90 | 50.30 ± 10.19 | 52.46 ± 15.82 |
| LDL Cholesterol (mg/dL) | 122.56 ± 11.53 | 133.60 ± 10.68 | 128.04 ± 10.40 |
| Triglycerides (mg/dL) | 112.97 ± 62.23 | 123.90 ± 58.60 | 125.54 ± 59.02 |
| HbA1c (%) | 5.27 ± 0.50 | 5.77 ± 0.52 | 5.56 ± 0.44 |
| 17-ß-Estradiol (pg/mL) | 27.23 ± 8.29 | 29.17 ± 8.61 | 27.45 ± 10.57 |
| Prolactin (ng/mL) | 8.59 ± 4.66 | 8.64 ± 3.43 | 9.23 ± 3.52 |
| Testosterone (ng/mL) | 4.97 ± 2.50 | 4.13 ± 1.64 | 4.59 ± 1.29 |

NA-ED: nonarteriogenic erectile dysfunction; A-ED: arteriogenic erectile dysfunction; BL-ED: borderline arteriogenic erectile dysfunction.
* $P < 0.048$ versus NA-ED.

The IIEF questionnaire [24] is a validated, self-administered tool, but we only evaluated the answers to the first five (erectile response dominium) of the fifteen questions (IIEF-15, 1–5) [24, 25]. Possible scores for the IIEF-5 range from 5 to 25; scores of 22–25 indicate normal erectile function while scores of 21 or below indicate ED [25]. Penile echo-color-Doppler was done in basal conditions and after intracavernous injection of 10 $\mu$g prostaglandin E1 (PgE1), and the peak systolic velocity (PSV) and end-diastolic velocity (EDV) were recorded 5, 10, 15, 20, and

25 min after the injection in the proximal portion of the penis. Patients were classified as "nonarteriogenic" (NA-ED) when their PSV was ≥35 cm/sec or ≤35 cm/sec but >25 cm/sec with concomitant EDV ≤0 cm/sec, "arteriogenic" (A-ED) when their PSV was ≤20 cm/sec, and "borderline" (BL-ED) with PSV between 25 and 21 cm/sec or with PSV between 35 and 25 with concomitant EDV >0 cm/sec [20–23, 26]. The erection quality was estimated 20 min after each injection. If a patient appeared stressed, he was given a second injection of the same dose of PgE1 and all measurements were repeated. Immediately before the penile echo-color-Doppler, participants were placed in a supine position and blood samples were drawn from a cubital vein. Samples were centrifuged at 3000 rpm, for 10 min. The serum was separated and stored at −80°C until analysis. All hs-TnT and hs-TnI were measured at the same time.

Serum concentrations of hs-TnT (Troponin T immunoassay; Roche Diagnostics, Switzerland) and of hs-TnI (Troponin I immunoassay; Johnson & Johnson, Italy) were measured according to the manufacturer's recommendations. The 99th percentile value for our normal reference population was 18.3 pg/mL for hs-TnT and 37 pg/mL for hs-TnI measured with a CV <10%.

All patients were referred to a cardiologist for evaluation of ischemic heart disease (IHD). The evaluation consisted of a comprehensive clinical history, physical examination, and an electrocardiogram while resting and during treadmill exercise. The diagnosis of ischemic heart disease relied on the detection of a 1 mm or more horizontal or downsloping ST-segment depression, frequent ventricular premature beats, or typical chest pain during the treadmill exercise test. In addition, the Duke Treadmill Score (DTS) as developed by Mark et al. [27] was calculated. The categories of risk based on the DTS are low risk (DTS > +5), moderate risk (DTS −10 to +4), and high risk (DTS < −11) [28].

All the results are expressed as the mean ± standard deviation. The data were not normally distributed and were normalized by using the log or log-log transformation. Undetectable values of hs-TnT and hs-TnI concentrations were considered equal to 0 for statistical purpose. In this regard, undetectable concentrations of hs-TnT were present in 49 out of 120 patients studied (40.8%) and undetectable concentrations of hs-TnI in 78 of the 120 studied patients (65.0%). The statistical analysis was carried out by using the ANOVA test taking into account the three groups of patients. The association between IIEF-5 and laboratory parameters was examined by Pearson's correlation coefficient. The estimate beta-power was equal to 0.004. $P$ value < 0.05 was considered significant.

## 3. Results

Of the 120 men included in the study, 40 patients (33.3%) were classified as having A-ED, 48 patients (40%) as having NA-ED, and, finally, 32 patients (26.6%) as having BL-ED. In A-ED (mean age 52 ± 8.5 years), NA-ED (mean age 58.1 ± 7.3 years), and BL-ED (mean age 50.0 ± 10.5 years) the mean of IIEF values was 12.8 ± 2.4, 10.5 ± 2.6, and 11.6 ± 1.8, respectively. There were no significant differences between the three groups ($P > 0.05$). All the men had DTS > +5 without significant difference between the three groups ($P > 0.05$).

All our data of hs-TnT and hs-TnI concentrations were in the reference range, as reported in Table 1. No significant difference ($P > 0.05$) of serum concentration of both hs-TnT and hs-TnI was found among the three groups of ED patients. The hs-CRP values were higher in A-ED men compared with NA-ED ($P = 0.048$) but not compared with BL-ED ($P = 0.136$), while no significant difference was found between BL-ED and NA-ED ($P = 0.964$). Spearman's correlation analysis demonstrated a negative correlation between serum hs-CRP and IIEF-5 that reached the significance in A-ED ($r = -0.480$; $P = 0.031$) but without reaching the significance in NA-ED and BL-ED [29, 30]. No statistically significant differences were found regarding the other laboratory parameters evaluated (i.e., creatinine, glucose, ALT, AST, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, 17-$\beta$-estradiol, prolactin, and testosterone) (Table 1).

## 4. Discussion

A consensus exists to consider ED an independent predictor of CVD [31–36], including CAD [37], peripheral artery disease [38], and stroke [39]. The early symptom of erectile difficulty often occurs before the development of structural, occlusive arterial disease and may therefore be one of the first signs of systemic vascular disease [14]. In other words, ED could be a sentinel marker for the presence of silent vascular disease in asymptomatic subjects. For this reason every effort should be made to check if cardiovascular risks are present in patients with ED or recognize when they begin to be present. Clinical studies revealed that the onset of ED symptoms occurs 2 to 3 years before CAD symptoms [40, 41] and 3 to 5 years before cardiovascular events [42, 43]. This relatively long time lag offers important potential in estimating and, ultimately, reducing cardiovascular risk in men with ED. However, it should be stressed that the penis is not always the most susceptible organ to inflammatory and atherosclerotic changes. Accordingly, although ED frequently precedes the onset of CAD, a considerable proportion of patients have CAD without concomitant ED, and vice versa, proving that the clinical course of atherosclerosis is multifaceted and not fully predictable. In diseases like ED, there is a need for a marker with a high negative predictive value for cardiac injury (ability to "rule out" the disease in patients with ED based on high sensitivity). In other words, it is imperative that a biomarker should ensure that ED cases at high risk of future cardiac events are not missed, because of the great benefit of treating these patients with a proven safe treatment. Cardiac troponins I and T are considered the most sensitive and specific biochemical markers of myocardial damage and the increased analytical sensitivity of the new generation methods demonstrated that measurable troponin was also present in the blood of almost 100% of healthy adults [44–46].

The aim of the study was to assess the presence of cardiovascular involvement with the measurement of both hs-TnT and hs-TnI levels in ED patients in order to begin the appropriate treatment quickly following the first symptoms to prevent significant cardiovascular events.

The populations studied were homogeneous without clinical evidence of atherosclerotic diseases; all men had normal ECG without ST-segment depression in exercise stress test and the DTS > +5.

Our results, using the measurement of hs-TnT or hs-TnI, at least in our experimental conditions, exclude the presence of cardiovascular injury in all patients with ED for less than one year and also in those of arteriogenic etiology. The fact that no differences were found among the three groups excludes the possibility of other statistical analyses such as the exploration of an optimal cut-point to differentiate the three groups.

In our patients we found that the serum concentrations of hs-CRP are higher in A-ED and the levels are associated with penile arterial disease severity assessed with IIEF-5. These data agree with those previously reported [29, 46–48]. In particular, plasma levels of hsCRP were found significantly higher in patients with ED and furthermore associated with penile arterial disease severity in men with ED without clinically apparent cardiovascular disease [30].

The number of patients included in this study seems to be low, but it must be considered that the population studied was carefully selected. In addition, the patients studied had no clinical evidence of atherosclerotic diseases and were free of the common risk factors associated with generalized penile arterial insufficiency such as hypertension, hyperlipidemia, cigarette smoking, diabetes mellitus, and pelvic irradiation. This is the first study to compare the value of troponin as a marker of cardiovascular disease before clinical symptoms in ED patients. Finally, we do not know whether troponin (T or I) may add information by adding the determination of other biomarkers such as NT-proBNP, as recently suggested [49, 50], or clinical variables such as echocardiography. Given the complementary and independent prognostic value of various markers, a "multimarker" approach in men with ED may be an effective strategy to improve prediction of cardiovascular risk beyond the use of traditional risk factors in daily clinical practice.

In conclusion, this study shows that the measurement of hs-TnT or hs-TnI levels allows us to exclude the presence of cardiovascular disease in patients with ED. The reason is probably the short history of ED, which was of less than one year. Longitudinal study is currently in progress to follow the level of hs-Tn (I and T) in this selected group of men, particularly in A-ED, in order to recognize when the heart begins to be affected by the process of atherosclerosis, before clinical symptoms. At present, we know the basal levels of both hs-Tn types in all our patients, and knowing the reference change values (RCV) [51–54], we can recognize when the level will increase over the RCV, even if it remains within the reference interval. Knowing that a minimum clinical change if present would indeed be diagnostically useful.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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*Review Article*

# Prognostic Value of Galectin-3 in Patients with Heart Failure

**Ivica Bošnjak,[1] Kristina Selthofer-Relatić,[1,2] and Aleksandar Včev[2,3]**

[1]*Department for Cardiovascular Disease, Clinic for Internal Medicine, University Hospital Centre Osijek, J. Huttlera 4, 31000 Osijek, Croatia*
[2]*Faculty of Medicine Osijek, University Josipi Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, Croatia*
[3]*Department for Gastroenterology, Clinic for Internal Medicine, University Hospital Centre Osijek, Osijek, Croatia*

Correspondence should be addressed to Ivica Bošnjak; dribosnjak79@gmail.com

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Galectins are a family of soluble $\beta$-galactoside-binding lectins that have important role in inflammation, immunity, and cancer. Galectin-3 as a part of this lectin family plays a very important role in development of heart failure. According to recent papers, galectin-3 plasma level correlates with heart failure outcome, primarily with rehospitalisation and death from heart failure. This paper summarizes the most recent advances in galectin-3 research, with the accent on the role of galectin-3 in pathophysiology of myocardial remodelling and heart failure development—with preserved and reduced ejection fraction, and some implication on development of new disease modifying drugs.

## 1. Introduction

Heart failure (HF) can be defined as a complex mechanical and neurohumoral syndrome manifested by hemodynamic congestion presenting with dyspnea, orthopnea, paroxysmal nocturnal dyspnea, or peripheral oedema, along with the presence of objective evidence of cardiac dysfunction [1, 2]. A new symptom in heart failure, bendopnea, has been described recently. Bendopnea is mediated via a further increase in filling pressures during bending when filling pressures are already high, particularly if cardiac index is reduced [3]. Heart failure remains one of the most prevalent and challenging medical conditions. Despite advances in treatment, morbidity and mortality in HF are very high, thus representing one of the most costly medical conditions [4]. Until now, HF has been considered to be associated with impaired cardiac contractility and cardiac dilation, but it has become evident that a considerable portion of patients presenting with clinical HF have a normal ejection fraction (EF). Now we can distinguish heart failure with reduced ejection fraction < 40% (HFrEF) and heart failure with preserved ejection fraction > 40% (HFpEF) [5, 6]. The pathophysiology concept of HF is related to the concept of myocardial remodelling. Increased stress or injury to the myocardium due to hypertension, diabetes mellitus, or ongoing ischemia can contribute to cardiac remodelling [7]. Response to acute or chronic damage can involve activation of immune cells to the myocardium, production of cell signaling proteins from local pericytes, mast cells, and macrophages, resulting in activation of resident fibroblasts and myofibroblasts, and deposition of collagen into extracellular matrix, which is correlated with collagen generating cardiac fibrosis [8]. There are many regulators involved in the pathophysiology of cardiac fibrosis. One of them is galectin-3.

## 2. Galectin-3 and Myocardial Remodelling

Galectin-3 (Gal-3) is a member of a galectin family involved in numerous physiological and pathological processes, some of which, inflammation and fibrosis, are pivotal contributing pathophysiological mechanisms to the development of HF. Gal-3 is a 29–35 kDa chimaera-type galectin which is unique in that it is the only member of the galectin family with an extended N-terminal domain constituted of tandem repeats of short amino acid segments linked to a single C-terminal carbohydrate-recognition domain. C-terminal domain is responsible for lectin activity, while the

presence of the N-terminal domain is necessary for full biological activity of galectin-3 [9, 10]. It is found in a wide range of tissues [11]. Gal-3 lacks a secretion signal peptide for classical vesicle-mediated exocytosis, so it is localized primary not only in the cytoplasm but also in the nucleus of mitochondria. When secreted in the extracellular space Gal-3 can interact with cell surface receptors to initiate transmembrane signalling pathways for different cellular functions. Gal-3 is also necessary for normal macrophage function [12]. In granulocyte-macrophage colony-stimulating factor transgenic mice, the expression of Gal-3 was increased by 6-fold following the activation of macrophages [13]. Significant infiltration of macrophages was observed in hypertrophied heart, as well as in active myocarditis, and colocalized Gal-3 was found [1, 14]. Consequently, it can be concluded that Gal-3 has dependent stimulatory effect on macrophage migration. This hypothesis was confirmed by Sharma et al. by showing that exogenous recombinant Gal-3 significantly increased macrophage migration, an effect that was inhibited by the endogenous peptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) [15]. Henderson et al. found that macrophages had abundant Gal-3 within their nucleus and cytoplasm and that they were able to secrete substantial amounts of Gal-3 into the supernatant in the cell culture suggesting a location in the extracellular space [16]. In a murine model of hepatic fibrosis, Gal-3 was found to be localized by proliferating fibroblasts [17]. In normal rat cardiac fibroblasts, addition of exogenous recombinant Gal-3 significantly increased fibroblast proliferation, which resulted in an increased collagen production in hypertrophied heart [15]. Simultaneously, Gal-3 binding sites were localized around the nucleus of proliferating fibroblast, whereas resting cells only had minimal binding sites in the cytoplasm [18]. It was suggested that Gal-3 induced cardiac fibrosis via activation of cyclin D1, thus allowing a macrophage derived mediator to affect the myocardium [18]. Similarly, involvement of Gal-3 in development of fibrosis has been demonstrated not only in the heart [18] but also in the liver and in the kidney [16, 17].

Cardiac remodeling is a crucial element in the clinical outcome of heart failure, and therefore various drugs are administered to prevent ongoing damage to the heart. Sharma and colleagues showed that Gal-3 was the strongest differentially regulated gene by comparing compensated and decompensated left ventricular hypertension in rats [18, 19]. The assumption that galectin-3 was one of the causes involved in the onset of heart failure was corroborated by infusing Gal-3 into the pericardial sac of wild type rats, which prompted extensive myocardial fibrosis [18, 19]. Other authors had similar results in murine model (angiotensin II-infused mice). Sharma et al. showed that coinfusion of Ac-SDKP along with galectin-3 into the pericardium may play a significant role in cardiac remodeling, not only in inhibiting fibrosis and inflammation but also in alleviating cardiac dysfunction [15].

Fibrosis and scar formation are a part of maladaptive mechanism to the injury. Fibroblasts and myofibroblasts as well as macrophages have been identified as key cells in development and growth of tissue scarring [20, 21]. Upregulation of Gal-3 was found in different human fibrotic conditions (liver cirrhosis, idiopathic lung fibrosis, and chronic pancreatitis) [17, 22–24]. Animal models of hepatic, renal, and cardiac fibrosis have also demonstrated the upregulation of Gal-3 [16, 17, 24]. Galectin-3 mRNA expression was significantly correlated with the extent of fibrosis. Inflammation is essential for tissue healing and scar formation. Sustained inflammation can lead to formation of extensive scar tissue and consequently to organ failure. Macrophages are a key cell type in development of fibrosis [25–27]. Specific depletion of macrophages significantly reduced myofibroblasts activation and decreased fibrosis (characterized by reduced alpha-SMA and collagen expression) [16].

The same authors demonstrated that disruption of Gal-3 gene did not affect macrophage recruitment or macrophage proinflammatory cytokine profiles in response to interferon $\gamma$ [16]. On the other hand, complete macrophage depletion in the Ren-2 rat model accelerated cardiac remodeling, supporting the notion that macrophages have a crucial role in remodelling [28]. We can conclude that galectin-3 and macrophages are major mechanisms in myofibroblast accumulation and activation, as well as in final fibrosis development.

## 3. Galectin-3 and Heart Failure with Reduced Ejection Fraction

The maladaptive changes that occur in surviving myocytes and in the extracellular matrix after myocardial injury lead to pathologic remodelling of the left ventricle, with dilatation and impaired contractility [29]. If these changes are left untreated, they worsen over time, exacerbated by additional injury and by systemic responses to left ventricular systolic dysfunction, notably activation of the sympathetic and renin-angiotensin-aldosterone systems [30, 31]. All these responses have detrimental systemic effects, accounting for the clinical manifestations of the syndrome of heart failure, including the development and worsening of symptoms, declining functional capacity, episodes of frank decompensation that result in the need for hospitalization, myocardial electrical instability, and premature death, usually due to pump failure or a ventricular arrhythmia [29]. Since the limited cardiac reserve of patients with systolic heart failure depends on atrial contraction and synchronized contraction of the left ventricle, events that affect these functions or that impose an additional hemodynamic load on the failing heart can lead to acute deterioration. Interruption of left ventricular remodeling and of the systemic responses to it is the basis of much of the effective treatment of heart failure [29].

The majorities of patients with HF have coronary artery disease and ischemic cardiomyopathy with zones of myocardial fibrosis as a respond to myocardial ischemia and infarction. Dilated cardiomyopathy may also result from a genetic cause, previous viral infection (recognized or unrecognized), alcohol abuse, or, occasionally, chemotherapy [29].

Increased stress and injury of the myocardium can contribute to myocardial remodeling [7]. Respond to myocardial damage involve recruitment of immune cells to the myocardium and production of signaling proteins from

local pericytes, mast cells, and macrophages, resulting in activation of resident fibroblast and deposition of procollagen into extracellular matrix which leads to cardiac fibrosis. In the myocardium, aldosterone is a major stimulus for macrophages secretion of galectin-3, which in turn works as a paracrine signal on fibroblast to help translate the signal of transforming growth factor-$\beta$ to increase cyclin D1 and direct the proliferation of myofibroblast and collagen deposition [32]. Galectin-3 is the most upregulated protein in an animal model of left ventricular hypertrophy and HF [15]. Recombinant galectin-3 induced cardiac fibroblast proliferation, collagen production, and cyclin D1 expression. The same investigators demonstrated that intrapericardial infusion of galectin-3 into healthy rats increased left ventricle collagen density and reduced ejection fraction of left ventricle for 22%. These data strongly suggest that galectin-3 is crucial for development of HF [15, 33]. Progressive cardiac fibrosis is central aspect of progressive systolic heart failure leading to creating tissue heterogeneity and stiffness that can cause sudden cardiac death by malignant arrhythmias [29, 32].

## 4. Galectin-3 and Heart Failure with Preserved Ejection Fraction

Diastolic dysfunction or heart failure with preserved ejection fraction (nonsystolic HF) represents an abnormality of diastolic distensibility, filling, or relaxation of the left ventricle, regardless of whether ejection fraction is normal or abnormal and whether the patients are with or without symptoms [30]. HFpEF has a different prognosis and treatment approach than HFrEF. Nonsystolic HF poses a challenge to diagnose with imaging modalities and the set of associated comorbidities such as advanced age, renal disorders, and diabetes [34]. It is known that angiotensin II directly and via stimulation of aldosterone is a crucial neurohormone involved in pathogenesis of cardiac fibrosis and impaired myocardial relaxation [34].

Zile et al. had demonstrated in one small series that galectin-3 levels were significantly elevated in cohort of patients with HFpEF [35]. Galectin-3 might provide an early warning marker for patients who are at risk for development of HF symptoms and may allow medical intervention. According to other animal and human studies, galectin-3 in addition to clinical and echocardiographic parameters can be used to confirm the presence of impaired diastolic function. Some studies have shown that galectin-3 had independent prognostic value, even after correction for established risk factor such as age, sex, BNP level, renal function, and diabetes mellitus [36]. Prognostic value of galectin-3 levels in plasma appears to be much stronger in the subset of patients with HFpEF in comparison with HFrEF [37]. Also, base line levels of galectin-3 seem to be sufficient to predict outcome, because serial measurement did not increase the prognostic yield [36].

## 5. Galectin-3 as Biomarker

Until recently, the goal of HF treatment was based on symptomatic relief. According to new trials and knowledge of myocardial remodelling as a crucial factor in HF development, slowing or reversing the progression of the disease is recognized as important goal of novel therapy (e.g., inhibition of angiotenzin-renin-aldosteron system) [36]. Identifying Gal-3 as an important segment in development of both myocardial remodelling and heart failure has opened possibility of Gal-3 being used as a new marker for the disease prognosis as well as a new treatment target.

Brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) assay were a golden standard in prognosis of HF in the past years. Natriuretic peptides are relisted by the myocardium as a result of myocardial stretching. In normal condition their level vary widley. In heart diseases condition, it is a marker of worsening heart failure and can present practical tool for heart failure treatment [37]. Troponin T and Troponin I (TnI/TnT) assay is useful in HF patients and according to trial data, slight elevations or chronically elevated levels of TnI/TnT predict a poor outcome [38]. Raised TnI/TnT levels are highly specific for myocyte injury. They are primarily used as markers in acute myocardial infarction, as well as in heart failure and some other conditions [39]. Like NTproBNP/BNP, the circulating TnI/TnT level is not pathogenic and specific and can be viewed as a signal of an ongoing pathological process in the heart. On the other hand, Gal-3 complements other HF biomarkers by providing an upstream signal of the myocardial fibrotic state, ventricular adverse remodelling, and progression of cardiomyopathy. Considering that cardiac fibrosis is irreversible process, Gal-3 measurement provides serological overview of the ongoing fibrotic process. BNP/NT-proBNP, TnT/TnI, and Gal-3 aid in prognosis, risk stratification, and management. Gal-3 levels are a direct reflection of cardiac fibrosis, are not acutely changed by HF decompensation, stay elevated once elevated in majority of cases, and are not affected by medical treatment [40]. DEAL-HF trial showed that plasma galectin-3 level has a prognostic value regardless of heart failure severity, as assessed by NT-proBNP levels, and it may be potentially used in management of such patients [41]. Galectin-3 and its prognostic value have been evaluated in number of studies. In 240 patients with stable chronic HF plasma Gal-3 levels were strongly related to outcome [42]. In another trial, data for 599 patients presented with dyspnoea at the emergency department were analyzed by receiver operating characteristic analysis. The results showed that in two-month period mortality was higher in patients with higher plasma galectin-3 level, presenting with a greater area under the curve at 0.74 compared with NT-proBNP [37]. Multivariate logistic regression analysis revealed that elevated plasma galectin-3 level was the best independent predictor of 60-day mortality or combination of death/recurrent HF within 60 days. Milting et al. found significantly elevated plasma galectin-3 levels at the time of mechanical circulatory support [43]. Patients who died had significantly higher plasma Gal-3 level than those who were transplanted. This is also one more argument that galectin-3 plays an important role in myocardial remodelling and HF development. Galectin-3 is also associated with the risk of developing HF after acute coronary syndrome and supports potential clinical relevance

of galectin-3-related pathway in patients with ischemic heart disease [44]. Galectin-3 was a strong independent predictor of 30-day major adverse cardiac outcome among patients with STEMI infarction undergoing primary PCI and thus can be utilized as a useful biomarker for stratifying high and low risk subgroups in daily clinical practice [45].

As mentioned before, various fibrotic conditions are associated with upregulation of galectin-3. Not only heart fibrosis but also upregulation of galectin-3 has been described in animal model for hepatic and renal fibrosis; in human liver cirrhosis; and in idiopathic lung fibrosis and chronic pancreatitis [16, 17, 46–48]. When taking into consideration importance of galectin-3 in heart failure pathophysiology, certainly we have to exclude the impact of these conditions on the final conclusions. Despite the fact that the frequent companion of heart failure is cardiorenal syndrome (renal failure), after correction for established risk factor (diabetes, age, sex, and renal function) it has been proven that high levels of galectin-3 has independent and significant impact on the prognosis of patients with heart failure [36, 49].

## 6. Conclusion

In every day clinical practice Gal-3 can be used to identify those patients at highest risk for readmission or death of HF. Galectin-3 measurement may be a significant factor in making a decision regarding visit intervals of whom to admit in patients with worsening HF. Due to the fact that Gal-3 levels are directly correlated with remodelling and fibrotic process in the myocardium, Gal-3 can be used as a culprit biomarker and can contribute to heart failure treatment as a potential novel target in therapeutics. This would be a real disease-modifying therapy to inhibit the remodelling process or slow down HF progression. Galectin-3 may one day allow identification and treatment of patients with coronary artery disease with a major risk of cardiomyopathy development.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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*Research Article*

# NT-proBNP as Early Marker of Subclinical Late Cardiotoxicity after Doxorubicin Therapy and Mediastinal Irradiation in Childhood Cancer Survivors

**Amal Zidan,[1] Laila M. Sherief,[2] Amera El-sheikh,[1] Safaa H. Saleh,[2] Doaa A. Shahbah,[2] Naglaa M. Kamal,[3] Hanan S. Sherbiny,[2] and Heba Ahmad[1]**

[1]*Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt*
[2]*Pediatric Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt*
[3]*Pediatric Department, Faculty of Medicine, Cairo University, Cairo 11562, Egypt*

Correspondence should be addressed to Laila M. Sherief; lamesh25@yahoo.com

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*Background*. Childhood cancer survivors treated with anthracyclines and mediastinal irradiation are at risk for late onset cardiotoxicity. *Aims of the Study*. To assess the role of N-terminal pro-brain natriuretic peptide (NT-proBNP) and tissue Doppler imaging (TDI) as early predictors of late onset cardiotoxicity in asymptomatic survivors of childhood cancer treated with doxorubicin with or without mediastinal irradiation. *Methods*. A cross-sectional study on 58 asymptomatic survivors of childhood cancer who received doxorubicin in their treatment protocols and 32 asymptomatic Hodgkin's lymphoma survivors who received anthracycline and mediastinal irradiation. Levels of NT-proBNP, TDI, and conventional echocardiography were determined. Results. Thirty percent of survivors had abnormal NT-proBNP levels. It was significantly related to age at diagnosis, duration of follow-up, and cumulative dose of doxorubicin. TDI detected myocardial affection in 20% more than conventional echocardiography. Furthermore, abnormalities in TDI and NT-pro-BNP levels were more common in Hodgkin lymphoma survivors receiving both chemotherapy and radiotherapy. *Conclusions*. TDI could detect early cardiac dysfunction even in those with normal conventional echocardiography. Measurement of NT-proBNP represents an interesting strategy for detecting subclinical cardiotoxicity. We recommend prospective and multicenter studies to validate the role of NT-proBNP as an early marker for late onset doxorubicin-induced cardiotoxicity.

## 1. Introduction

Anthracyclines are widely used antineoplastic agents for the treatment of both childhood hematological malignancies and solid tumors, including acute lymphoblastic leukemia (ALL), acute myeloid leukemia, non-Hodgkin and Hodgkin's lymphoma, neuroblastoma, osteosarcoma, Ewing tumors, and nephroblastoma. Almost 60% of children with cancer receive anthracyclines as a part of their treatment [1]. A major limitation of anthracycline is the risk of cardiotoxicity, manifested as asymptomatic cardiac dysfunction in up to 57% [2, 3] and cardiomyopathy with subsequent clinical heart failure in up to 16% [4]. Subclinical cardiac abnormalities are persistent and progressive after anthracycline therapy and can lead to significant clinical symptoms [5]. Early and accurate diagnosis of ventricular dysfunction in asymptomatic cardiac patients may permit a prompt onset of therapy of subclinical cardiotoxicity before the development of life-threatening complication [6].

Current monitoring techniques, such as MUGA (multi-gated acquisition scan) or echocardiography, have substantial limitations and detect LV dysfunction only after it had occurred. Cardiotoxicity is usually diagnosed only upon manifestation of clinical signs and symptoms or progressive cardiac dysfunction. Thus, new diagnostic tests are required to confirm ventricular dysfunction induced by anticancer therapy [6]. It has been suggested that NT-proBNP may be useful in early detection of myocardial damage after anti-cancer therapy [7–9]. However, few data have been published demonstrating the usefulness of NT-proBNP in detection

of late onset cardiotoxicity occurring several years after completion of chemotherapy in childhood cancer survivors [10].

This study aimed to assess the role of N-terminal pro-brain natriuretic peptide (NT-proBNP) and tissue Doppler imaging (TDI) as early predictors of late onset cardiotoxicity in asymptomatic survivors of childhood cancer treated with doxorubicin with or without mediastinal irradiation.

## 2. Methods

This cross-sectional study was carried out on 80 asymptomatic survivors of childhood cancer, who visited the late effects clinics of Pediatric Oncology, Zagazig University Hospitals, and late effects clinics of Pediatric Oncology, Benha Specialized Pediatric Hospital, during the period from January 2011 to December 2013. All children received doxorubicin as a part of their therapy for various kinds of malignancy for more than one year. Informed consent was obtained from the patients or their parents. Our inclusion criteria were patients in a stable general condition with no signs or symptoms of cardiac impairment at the time of evaluation and normal hepatic and renal function tests.

Patients with history of cardiac diseases and hypertension were excluded from the study. The study was approved by the local ethics committee of contributing hospitals.

All survivors underwent all the following.

(1) Complete history taking and thorough physical examination including weight, body surface area (BSA), and measurement of blood pressure.

(2) The cumulative dose of anthracycline calculated for each patient according to his BSA.

(3) Methods for evaluation of subclinical cardiotoxicity.

*(a) Conventional Echocardiography.* Detailed conventional echocardiography was performed at Pediatric Cardiology Unit, Zagazig University Hospitals, using (Vivid 7 Pro, 7 MHz and 3 MHz transducer, GE, Horten, Norway). Echocardiography was performed by a cardiologist who was blinded to the clinical details. Two-dimensional, M-mode, pulsed-wave (PW), and continuous-wave Doppler echocardiographic images were acquired. For all patients, standard measurements were left ventricular posterior wall thickness at diastole (LVPW), interventricular septum thickness at diastole (IVS), left ventricular dimensions at end-systole (LVES), and left ventricular dimensions at end-diastole (LVED). Shortening fraction (FS) and ejection fraction (EF) of the LV were calculated from M-mode measurements of LV dimensions at the level of mitral valve leaflets in parasternal long-axis view. Sample volume of the PW Doppler was placed between the tips of the mitral leaflets in the apical four-chamber view, and then diastolic functions of the left ventricle were measured (peak early [$E$] and late [$A$] diastolic wave velocities of the mitral valve as well as $E/A$ ratio). All measurements were compared with the normal values of LVPW, IVS, LVES, and LVED which were taken from the standard tables according to ages and BSA [11]. An EF of less than 55% and FS of less than 29% were considered abnormal [12].

*(b) Tissue Doppler Imaging (TDI).* TDI was obtained according to the methods described by Kapusta et al. [13]. TDI studies were performed by the same echocardiography device. Apical four-chamber views were obtained and longitudinal peak annular velocity ratio was measured at lateral annulus of the mitral valve (pulsed tissue Doppler mode).

In the parasternal, long-axis view measurements of peak myocardial velocities were made guided by color coded TDI. Peak myocardial velocity during systole ($S'$), early diastole ($E'$), and late diastole ($A'$) was measured at the right and left ventricular free walls (RVFW and LVFW), respectively. Peak longitudinal myocardial velocities ($S'$, $E'$, and $A'$) were assessed within basal, middle, and apical parts of the right ventricular anterior wall and left ventricular posterior wall.

*(c) NT-proBNP Analysis.* Venous blood samples were obtained from an indwelling catheter after 30 minutes of rest in supine position. The blood samples were withdrawn into chilled tubes containing EDTA. The whole blood was centrifuged; plasma was decanted, immediately frozen, and stored at −27°C until assayed (within 6 months after sampling). Plasma concentration of NT-proBNP was measured by electrochemiluminescence immunoassay. It was performed with Modular $E$, using the NT-proBNP (Roche Diagnostics, Mannheim, Germany). The abnormal level was defined in our survivors based on age-dependent reference values by Albers and his colleagues [14].

*2.1. Statistical Analysis.* Data were analyzed using Microsoft Office 2007 (Excel) and Statistical Package for Social Science (SPSS) version 19.0.0, SPSS Inc., Chicago, IL, USA. Data were summarized using the arithmetic mean, standard deviation (SD), screening test, Student's $t$-test, Chi-square test ($\chi^2$), and Mann-Whitney $U$ test. Probability ($P$) value was considered for statistical significance if it was less than 0.05.

## 3. Results

*3.1. Subjects Characteristics.* Our study included 80 children who were treated for childhood cancer and received anthracycline chemotherapeutic agents in their treatment protocols. None of the survivors had a history of acute cardiotoxicity following anthracycline dosage. Males and females were equally distributed, and the mean age at diagnosis was 5.62 + 1.72 years, while the mean duration of follow-up was 3.94 ± 1.37 years. The range of cumulative anthracycline doses was 175–380 mg/m$^2$. Thirty-two patients (40%) had Hodgkin lymphoma, 27 patients (33.75%) had non-Hodgkin's lymphoma, and 21 patients (26.25%) had solid tumors (Wilms' tumor, neuroblastoma, and Rhabdomyosarcoma) (Table 1).

*3.2. NT-proBNP Level.* In the present study, 24 patients representing 30% of the studied children showed abnormally high levels of NT-proBNP. There was no significant relation between abnormal NT-proBNP levels and the gender of patients. However, abnormal NT-proBNP levels were significantly related to age at diagnosis, duration of follow-up, and cumulative anthracycline dosage ($P < 0.001$). Abnormal NT-proBNP levels were associated with younger age of

Table 1: Characteristics of 80 asymptomatic survivors of childhood cancer.

| | |
|---|---|
| Total number of study population | **80** |
| Gender | |
| Male | 40 |
| Female | 40 |
| Age at diagnosis (years) | |
| Mean ± SD (range) | 5.62 ± 1.72 (3–11) |
| Duration of follow-up (years) | |
| Mean ± SD (range) | 3.94 ± 1.37 (2–7) |
| CAD (mg/m$^2$) | |
| Mean ± SD (range) | 245.4 ± 57.7 (175–380) |
| Diagnosis *n* (%) | |
| Hodgkin's lymphoma | 32 (40) |
| Non-Hodgkin's lymphoma | 27 (33.75) |
| Solid tumors | 21 (26.25) |
| Wilms' tumor | 11 (52.4) |
| Neuroblastoma | 7 (33.3) |
| Rhabdomyosarcoma | 3 (14.3) |

CAD: cumulative anthracycline doses.

patients, longer duration of follow-up, and higher cumulative anthracycline doses (Figures 1, 2, and 3).

*3.3. Echocardiographic Parameters and NT-proBNP.* Echocardiographic parameters were obtained from all 80 survivors. Twelve patients (15%) had abnormal echocardiography. Abnormal left ventricular systolic function (EF and FS) and diastolic function ($E$ velocity and $E/A$ ratio) were not significantly related to abnormal NT-proBNP levels. $E$ velocity was 115 ± 15 cm/s in abnormal NT-proBNP group versus 110 ± 13 cm/s in normal NT-proBNP group ($P$ value > 0.05). $E/A$ ratio was 1.91 ± 0.31 in abnormal proBNP group versus 1.83 ± 0.21 in normal proBNP group ($P$ value > 0.05).

*3.4. Tissue Doppler Imaging and NT-proBNP.* Abnormal tissue Doppler imaging was diagnosed in 28 (35%) of the studied children.

Abnormal diastolic functions by TDI ($E/E'$) were positively correlated with cumulative dose of anthracyclines (Figure 4) and duration of follow-up and negatively correlated with age of patients.

There was a significant difference in LV diastolic function by pulsed tissue Doppler at lateral mitral annulus between normal and abnormal NT-proBNP groups. $E'$ (cm/s) was 8.18 ± 0.7 15 in abnormal NT-proBNP group versus 8.6 ± 0.6 in normal NT-proBNP group ($P$ value 0.007) and $E/E'$ was 14.06 ± 0.97 in abnormal NT-proBNP group versus 12.79 ± 1.03 in normal NT-proBNP group ($P$ value < 0.0001) (Figure 5).

There was a significant relation between abnormal NT-proBNP and peak myocardial velocities of right ventricle (RV) free wall using color coded TDI. Patients with increased level of NT-proBNP had significantly low systolic ($S'$) (Figure 6) and diastolic ($E'$ and $A'$) velocities in all right ventricular free wall segments with $P$ value < 0.001 in most

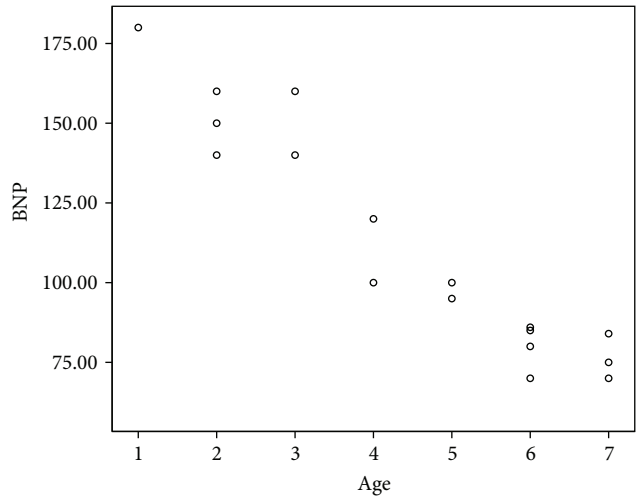


Figure 1: The correlation between NT-proBNP and age of patients.

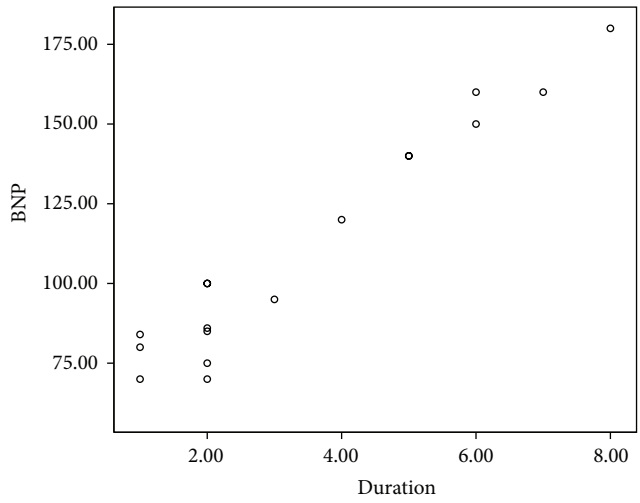


Figure 2: The correlation between NT-proBNP and duration of follow-up.

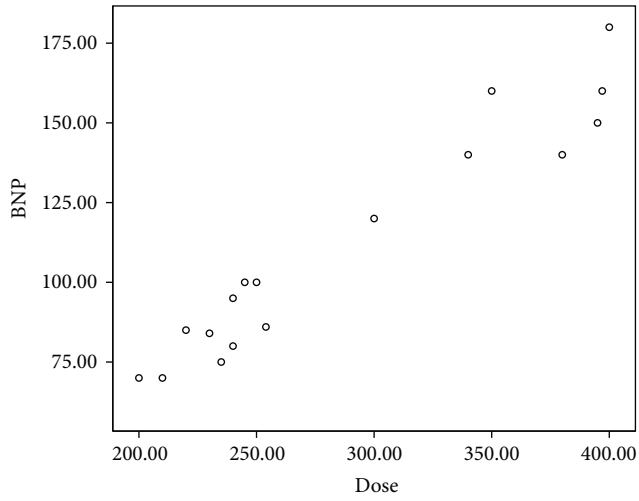


Figure 3: The correlation between NT-proBNP and cumulative dose of anthracycline.

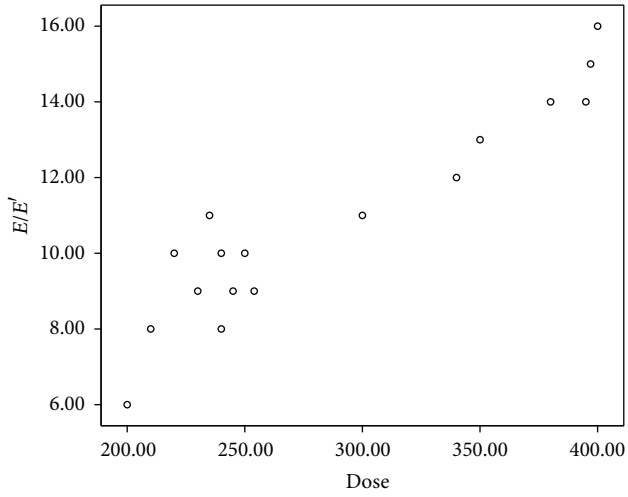


Figure 4: The correlation between diastolic function by TDI functions ($E/E'$) and cumulative dose of anthracycline.

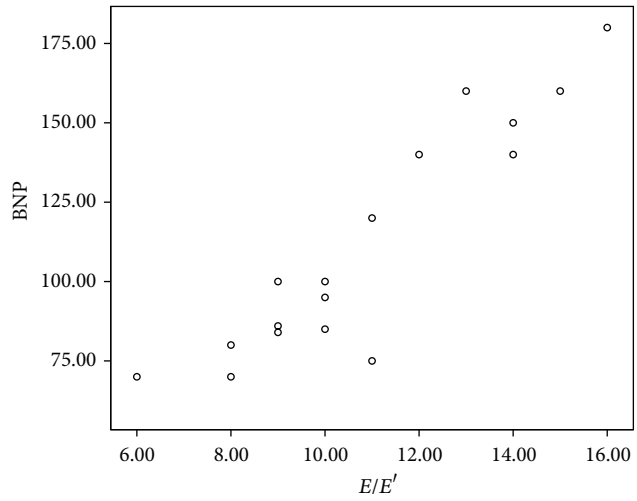


Figure 5: The correlation between proBNP and diastolic functions ($E/E'$).

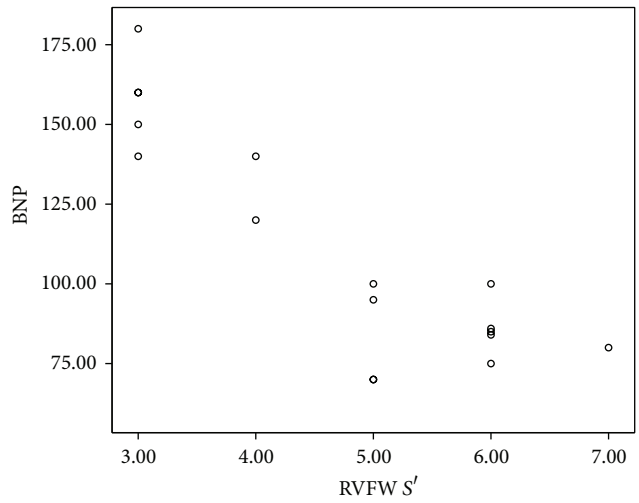


Figure 6: The correlation between NT-proBNP and RV systolic functions by TDI functions ($S'$).

of them. There was a significant relation between abnormal NT-proBNP and myocardial velocities of LV posterior wall. $S'$ (Figure 7) and $A'$ velocities of the LV posterior wall were significantly lower in apical, middle, and basal parts. $E'$ velocity was significantly low in mid segment of LV posterior wall in abnormal proBNP group versus normal proBNP group ($P$ value < 0.001); meanwhile there was no significant difference in $E'$ velocity of apical and basal parts of LV posterior wall between both groups (Table 2).

Our data showed a significant relationship between NT-proBNP and TDI parameters as all patients with increased serum level of NT-proBNP showed abnormalities in TDI, while only 4 out of 56 patients with normal level of NT-proBNP had abnormal TDI findings. None of patients with normal TDI showed high levels of NT-proBNP (Figure 8).

Furthermore, our patients were classified into an unexposed group (48 patients who received only chemotherapy) and exposed group (32 patients who received both chemotherapy and radiotherapy). Our data showed that the level of NT-proBNP was significantly higher in the exposed group when compared with the unexposed group. NT-proBNP level was 144.3 ± 28.6 pg/mL in exposed group versus 115 ± 13.7 pg/mL in unexposed group ($P$ value < 0.001). Regarding diastolic function assessment in study groups, there was no significant difference in early diastolic velocity by routine echocardiography between exposed and unexposed groups. $E$ velocity (cm/s) was 82 ± 10 versus 79 ± 11 in unexposed group ($P$ value 0.219); however there was a highly significant difference between both groups regarding diastolic function by TDI. $E'$ velocity (cm/s) was 5.7 ± 0. 8 in exposed group versus 8 ± 1.1 in unexposed group ($P$ value < 0.0001) and $E/E'$ ratio was 14.38 ± 0.92 in exposed group versus 9.87 ± 1.32 in unexposed group ($P$ value 0.0001) (Table 3). Compared to the unexposed group, all peak myocardial velocities ($S'$, $E'$, and $A'$) of different segments of LV posterior wall and RV anterior wall using color coded TDI were significantly lower in the exposed group (Table 4).

## 4. Discussion

Anthracycline chemotherapy and mediastinal and neck radiation are the most common causes of therapy-related cardiovascular complications in childhood cancer survivors [15]. The early identification of patients at risk for cardiotoxicity is a primary goal for both cardiologists and oncologists, allowing for the planning of personalized antineoplastic therapeutic strategies, the support of cardiac function, and the monitoring of the progression of cardiac damage [16]. There is a growing interest in the use of biomarkers for detection of anthracycline-induced cardiotoxicity. Natriuretic peptides are released by the myocardium in response to volume and pressure overload [17]. Although the role of NT-proBNP in the early detection of myocardial damage after anticancer therapy has been evaluated in several studies, the focus was mainly on level of this biomarker during or several

Table 2: Right and left ventricular TDI parameters in groups with normal and abnormal NT-proBNP.

| | Systole ($S'$) | | | Diastole ($E'$) | | | Diastole ($A'$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Abnormal $N = 24$ | Normal $N = 56$ | $P$ | Abnormal $N = 24$ | Normal $N = 56$ | $P$ | Abnormal $N = 24$ | Normal $N = 56$ | $P$ |
| RVFW a | 3.99 ± 0.81 | 5.03 ± 0.19 | <0.001 | 5.35 ± 1.52 | 7 ± 0.68 | <0.001 | 2.31 ± 0.7 | 3.22 ± 0.47 | <0.001 |
| RVFW m | 6.03 ± 1.7 | 7.2 ± 0.47 | <0.001 | 8.64 ± 1.88 | 10.4 ± 0.63 | <0.001 | 4.35 ± 1.41 | 6.67 ± 0.52 | <0.001 |
| RVFW b | 7.37 ± 1.62 | 8.75 ± 0.4 | <0.001 | 10.79 ± 2.2 | 12.1 ± 0,86 | <0.05 | 5.48 ± 1.1 | 7.14 ± 0.45 | <0.001 |
| LVFW a | 3.28 ± 0.5 | 3.89 ± 0.27 | <0.001 | 5.5 ± 0.95 | 5.7 ± 0.47 | NS | 2.31 ± 0.72 | 3.3 ± 0.32 | <0.001 |
| LVFW m | 4.27 ± 0.75 | 5.85 ± +0.62 | <0.001 | 7.6 ± 1.98 | 9.7 ± 0.58 | <0.001 | 3.18 ± 0.55 | 4.2 ± 0.56 | <0.001 |
| LVFW b | 5.6 ± 1.1 | 6.5 ± 1.03 | <0.05 | 10.48 ± 2 | 10.42 ± 0.8 | NS | 4.72 ± 0.75 | 5.31 ± 0.79 | <0.05 |

RVFW a, m, b: right ventricular free wall at the apical, middle, and basal parts, respectively.
LVFW a, m, b: left ventricular free wall at the apical, middle, and basal parts, respectively.
TDI: tissue Doppler imaging.

Table 3: Left ventricular diastolic function indices by echocardiography and pulsed TDI and NT-proBNP in exposed and unexposed study groups.

| | Unexposed group | Exposed group | $P$ value |
|---|---|---|---|
| NT-proBNP (pg/mL) | 115 ± 13.7 | 144.3 ± 28.6 | <0.001 |
| | Echo parameters | | |
| $E$ | 79 ± 11 | 82 ± 10 | 0.219 |
| $E'$ | 8 ± 0.11 | 5.7 ± 0.8 | 0.0001 |
| $E/E'$ | 9.87 ± 1.32 | 14.38 ± 0.92 | 0.0001 |

Systole, $E$: early rapid filling during diastole, $E'$: early diastole, and $A$: late diastole.

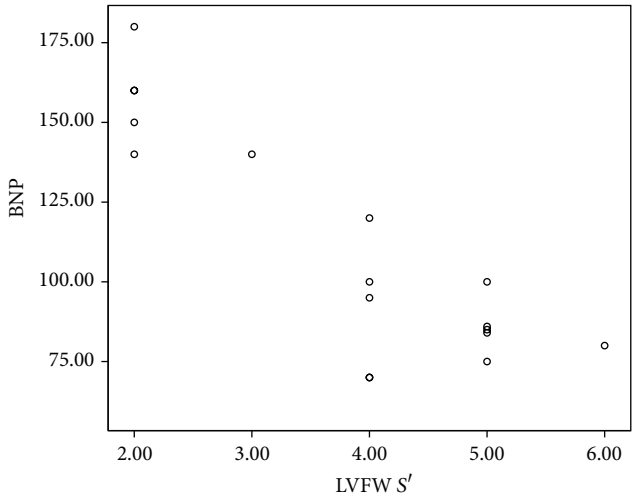


Figure 7: The correlation between NT-proBNP and LV systolic functions by TDI functions ($S'$).

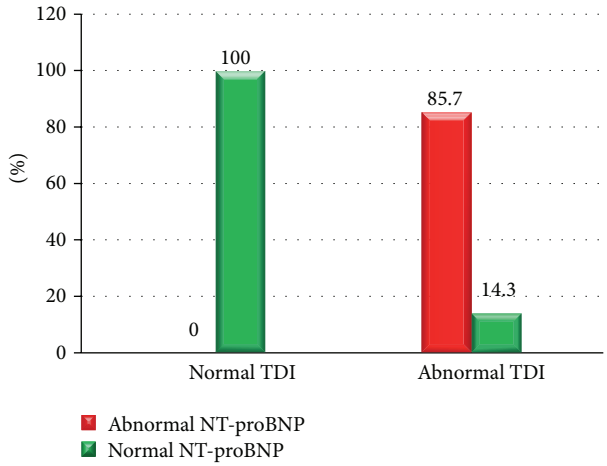


Figure 8: Relation between TDI and group with abnormal NT-proBNP and those with normal NT-proBNP.

months after therapy (7 and 18–22). In this study, NT-proBNP was used as a noninvasive technique for detection of late subclinical cardiotoxicity in survivors of childhood cancer. Abnormal NT-proBNP levels were detected in 30% of our asymptomatic survivors. This is in agreement with Mavinkurve-Groothuis et al. who found abnormal levels of NT-proBNP in 13% of 122 asymptomatic survivors of childhood cancer [18]. Moreover, the study of Sherief et al. detected an abnormal level of NT-proBNP in 20% of 50 of asymptomatic survivors [19]. Higher incidence in the current study may be related to the combined effects of anthracycline and radiotherapy as 40% of patients were exposed to both chemotherapy and radiotherapy. This result was in accordance with Adams and Lipshultz who demonstrated that concomitant cardiac irradiation has been recognized as risk factor of anthracycline cardiotoxicity [20]. Radiation may worsen the cardiotoxic effects of anthracycline, but whether this effect is additive or synergistic is unclear. Moreover, in the current study, the group exposed to both chemotherapy and radiotherapy showed higher levels of NT-proBNP than the unexposed group which was not exposed to radiotherapy. This is in agreement with D'Errico et al. who demonstrated increased level of NT-proBNP after radiotherapy when compared with nonirradiated patients [21]. Adams and Lipshultz demonstrated that concomitant cardiac irradiation has been recognized as a risk factor of anthracyclines cardiotoxicity [20]. This effect was explained by Braunwald who demonstrated that proinflammatory cytokines such as TNF and IL-6 were expressed in Reed-Sternberg cells from patients with Hodgkin disease (patients received chemotherapy and radiotherapy). These proinflammatory cytokines stimulate synthesis and secretion of NT-proBNP; at the same time they have been reported to impair cardiac function, several pathways are involved, including induction of apoptosis of cardiac myocytes by TNF type 1 and FAS activation [22]. Analysis of NT-proBNP in relation to risk factors of cardiotoxicity showed that abnormalities in NT-proBNP were significantly related to age at diagnosis, duration of follow-up, and cumulative anthracycline doses. The patients who had abnormal NT-proBNP were younger at diagnosis, had longer duration of follow-up, and received higher doses of anthracycline. The higher risks in the patients treated at younger age may be explained by immature cardiovascular tissues which are more vulnerable to chemotherapy and radiotherapy [23, 24]. These results were consistent with other authors [19, 25, 26]. On the contrary, Mavinkurve-Groothuis et al. found no significant relation between abnormal NT-proBNP levels and age at diagnosis and follow-up duration, but abnormal NT-proBNP levels were significantly related to cumulative anthracycline dosage [18]. Regarding the sex, our study showed no statistically significant difference regarding sex and NT-proBNP level. This result is in agreement with Mavinkurve-Groothuis et al. [18] but in disagreement with

Table 4: Right and left ventricular TDI parameters in exposed and unexposed study groups.

| | Systole | | | Diastole (*E*) | | | Diastole (*A*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Unexposed group | Exposed group | *P* | Unexposed group | Exposed group | *P* | Unexposed group | Exposed group | *P* |
| RVFW a | 5.02 ± 0.2 | 4.2 ± 0.87 | 0.000 | 5.03 ± 0.2 | 4.3 ± 0.86 | 0.001 | 5.06 ± 0.4 | 4.3 ± 0.52 | 0.000 |
| RVFW m | 7.2 ± 0.51 | 6.4 ± 1.67 | 0.018 | 6.69 ± 0.54 | 5.02 ± 1.52 | 0.000 | 6.69 ± 0.54 | 5.02 ± 1.52 | 0.000 |
| RVFW b | 8.9 ± 0.25 | 7.71 ± 1.5 | 0.000 | 7.27 ± 0.43 | 6.8 ± 0.42 | 0.000 | 7.27 ± 0.34 | 5.9 ± 1.2 | 0.000 |
| LVFW a | 3.8 ± 0.29 | 3.4 ± 0.51 | 0.001 | 3.41 ± 0.32 | 2.59 ± 0.79 | 0.000 | 3.41 ± 0.32 | 2.59 ± 0.79 | 0.000 |
| LVFW m | 5.57 ± 0.36 | 4.58 ± 0.86 | 0.000 | 3.9 ± 0.29 | 3.4 ± 0.64 | 0.002 | 3.9 ± 0.37 | 3.4 ± 0.64 | 0.002 |
| LVFW b | 7.07 + 0.44 | 5.9 ± 1.12 | 0.000 | 5.7 ± 0.48 | 4.9 ± 0.95 | 0.001 | 5.07 ± 0.48 | 4.58 ± 0.86 | 0.001 |

RVW a, m, b: right ventricular free wall at the apical, middle, and basal parts, respectively.
LVW a, m, b: left ventricular free wall at the apical, middle, and basal parts, respectively.
TDI: tissue Doppler imaging.
Exposed: to both chemotherapy and radiotherapy.
Unexposed: only chemotherapy.

that reported by Lipshultz et al. who detected sex-related differences in NT-proBNP levels and reported that female survivors are more vulnerable to anticancer cardiotoxicity [27]. Moreover, Bu'Lock et al. reported that girls are significantly at greater risk than boys for late depressed contractility even when receiving the same cumulative dose of doxorubicin [28]. The importance of continuing to follow children with, or at risk for, premature symptomatic cardiovascular disease cannot be overemphasized. With longer follow-up after anthracycline treatment, the prevalence and severity of cardiac abnormalities increase [29–31]. Similar results were reported in the current work. Thus, the importance of lifetime follow-up cannot be overstated and preventing late cardiotoxicity must be a research priority [31, 32], particularly as the number of asymptomatic cancer survivors at risk for cardiac dysfunction later in life increases. More than 6 years of follow-up is necessary to identify those long term survivors at risk for cardiac dysfunction [31].

One of the main risk factors for anthracycline cardiotoxicity is high cumulative dose which is associated with a higher incidence of subclinical cardiac dysfunction [33]. In the current study, an abnormal level of NT-proBNP was significantly related to increasing cumulative anthracycline dose. According to our information, other studies reported that abnormal levels of NT-proBNP were significantly related to cumulative anthracycline dose [6, 18, 34]. On the other hand, Barry et al. and Gianni et al. reported that no dose of anthracyclines is free of cardiotoxicity with increasing duration of follow-up [35, 36].

NT-proBNP has been shown to be a sensitive marker for heart failure in children in earlier studies [37–39]. In patients with cardiac dysfunction, NT-proBNP may be used for diagnosis, treatment monitoring, and prognosis implications [39, 40]. There have been few studies concerning the relationship between the serum NT-proBNP level and echocardiographic parameters. In the study conducted by Germanakis et al. higher NT-proBNP levels were associated with reduced LV mass in asymptomatic children treated with anthracyclines [41]. In other studies, abnormal NT-proBNP levels were found to be significantly related to the end-diastolic LV internal diameter [18, 41]. In their studies, Hongkan et al. and Pongprot et al. found a significant relation between the NT-proBNP and diastolic parameters [42, 43]. Meanwhile, Brouwer et al. reported that abnormal FS and/or abnormal diastolic functions were present in 43% of adult childhood cancer survivors. Their NT-proBNP levels were higher in association with increased wall motion score index [44]. Also Dorup et al. found a lower $E$ velocity in childhood cancer survivors exposed to anthracyclines [45]. In contrast to these studies, our study did not reveal any significant difference in systolic or diastolic function at the level of routine echocardiography between normal and abnormal NT-proBNP groups. This result is supported by Urbanova et al. [10] who could not reveal any echocardiographic changes in anthracycline treated patients with high NT-proBNP levels. Such differences between several studies are acceptable because there are various factors affecting the degree of cardiotoxicity, such as cancer type, cumulative anthracycline doses exposed, the age of the patient at the time of diagnosis, the time since the last chemotherapy, additional cardiotoxic medication, and history of mediastinal radiotherapy. Therefore, depending on the degree of myocardial injury, different correlations may be found between NT-proBNP levels and echocardiographic parameters.

TDI has been shown to be sensitive to identify anthracycline-induced cardiomyopathy and aberration in TDI parameters may be identified before abnormalities can be detected by conventional echocardiography [46]. In our study, we found that 35% of our survivors had global myocardial damage in the form of significant aberrations in peak myocardial velocities. Abnormal peak myocardial velocities were found in all cardiac cycles ($S'$, $E'$, and $A'$) in both LV and RV in some of our survivors in spite of normal routine echocardiography. Thus, TDI aberration was detected in hearts that appeared normal by routine echocardiography and might precede structural changes [46, 47]. Similar results were reported by Rathe et al. [48]. Aberration in TDI parameters has been shown to be highly sensitive to identify anthracycline-induced cardiomyopathy [46]. Moreover, all peak myocardial velocities ($S'$, $E'$, and $A'$ velocities) of different segments of LV posterior wall and RV free wall using color coded TDI were significantly lower in the group exposed to both anthracyclines and mediastinal irradiation when compared to the group exposed to anthracyclines only. This is in agreement with the study done by Adams and Lipshultz who demonstrated that concomitant cardiac irradiation has been recognized as a risk factor of anthracyclines cardiotoxicity [20].

Analysis of TDI parameters in relation to NT-proBNP showed a significant relation between elevated NT-proBNP levels and TDI abnormalities. Similar results were reported by Sherief et al. and Yildirim et al. [19, 49]. Tissue Doppler early diastolic velocity of the mitral annulus ($E'$) reflects the rate of myocardial relaxation, and it has been postulated as a good indicator of LV myocardial diastolic function. Normally, $E/E'$ at rest and during exercise ($E/E' < 8$) are similar. Decreased mitral annular $E'$ is one of the earliest markers of diastolic dysfunction. In early diastolic dysfunction, TDI mitral annular early diastolic function ($E'$) is disturbed, whereas $E$ velocity remains normal [50, 51]. In our study we found a significantly low $E'$ velocity in lateral mitral annulus with high $E/E'$ ratio in all cancer survivors with significantly higher $E/E'$ ratio in abnormal NT-proBNP group and the group exposed to combined chemotherapy and radiotherapy which suggest the role of TDI and NT-proBNP in early detection of late cardiotoxicity in childhood cancer survivors exposed to anthracyclines and in particular when combined with mediastinal irradiation. To the best of our knowledge, the only paper that studied the role of $E/E'$ ratio for detection of diastolic dysfunction in childhood cancer survivors exposed to anthracyclines was that done by Yildirim et al. who found a significant reduction in mitral septal annular early diastolic velocities $E'$ and elevation of mitral septal $E/E'$ values in a group exposed to anthracyclines [49]. However, Yildirim et al. rolled out patients exposed to mediastinal irradiation from the study [49], but we included them as a separate group, so, to the best of our knowledge, our study is the first to investigate the role of NT-proBNP

and TDI (both pulsed and color coded) for detection of late cardiotoxicity induced by anthracyclines and mediastinal irradiation in childhood cancer survivors. This suggests the clinical usefulness of proBNP and TDI in detection of anthracycline and radiotherapy-induced cardiotoxicity and highlights their role as early markers of cardiotoxicity induced by anthracycline and mediastinal irradiation. Although these methods are promising, they have not been validated as surrogate end points for clinical toxicity and they are not routinely performed. We recommend prospective and multicenter studies, including large populations, using well-standardized methods for NT-proBNP and well-defined timing of sampling and cardiologic end point to validate the role of NT-proBNP as an early marker for late onset doxorubicin-induced cardiotoxicity.

## Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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