



The evaluation of B-type Natriuretic Peptide and Troponin I in acute myocardial infarction and unstable angina

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ABSTRACT

Introduction: The diagnostic utility of B-type natriuretic peptide (BNP) has prompted interest in its use as an aid in the detection of early heart failure and assessment of diseases. The first objective of this study was measurement of BNP and troponin I (TnI) blood levels in patients with acute myocardial infarction (AMI) and unstable angina. The second objective of this study was to find a correlation between TnI and BNP in blood.

Methods: The concentrations of BNP and TnI in 150 blood levels were determined using CMIA (chemiluminescent microparticle immunoassay) Architect and 2000 (Abbott diagnostics). The retrospective study included 100 patients who were hospitalized at the Department of Internal Medicine of the University Clinical Center Sarajevo and 50 healthy control. The reference blood range of BNP is 0-100 pg/mL and TnI is 0.00-0.4 ng/mL.

Results: In the patients with AMI the mean value of BNP is 764.48 ± 639.52 pg/mL and TnI is 2.50 ± 2.28 ng/mL. The patients with unstable angina have BNP 287.18 ± 593.20 pg/mL and TnI 0.10 ± 0.23 ng/mL. Our studies have shown that the correlation between BNP and TnI was statistically significant for $p < 0.05$ using Student t test with correlation coefficient $r = 0.36$.

Conclusions: BNP and TnI levels can help to identify the patients with a high risk for cardiovascular diseases.

Keywords: BNP; TnI; acute myocardial infarction; unstable angina

INTRODUCTION

Since the discovery of the natriuretic peptides in the 1980s and their subsequent introduction into

clinical laboratory testing in the 2000s, assays of B-type Natriuretic peptides (BNP) have gained widespread acceptance as important tools for diagnosis and risk stratification in the acute-care setting (1,2). BNP was first isolated from porcine brain tissue, but heart has been determined to be the major source. It is synthesized and released in the blood in response to volume overload or conditions that cause ventricular stretch, to control fluid and electrolyte homeostasis by interaction with

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renin-angiotensin-aldosterone system. Pre-proBNP (134 amino acids) is synthesized in the cardiac myocytes and it is processed to a proBNP (108 amino acids) precursor molecule.

BNP is realized from cardiac myocytes due to their stretching, volume overload and high filling pressure (3-5). It is a neurohormone produced in the ventricular myocardium in response to dilatation and pressure overload, and its plasma concentration correlates with the magnitude of pressure and/or volume overload. As markers of neurohormonal activation, BNP and NT-proBNP were subsequently studied within clinical trials of acute coronary syndrome (ACS) as adjuncts to risk stratification and have been associated with short and long term mortality in (ACS) patients, even after adjusting for the presence of congestive heart failure (6,7). The levels of BNP increase with decreasing functional capacities and elevated levels in the patients with heart failure (HF) indicate disease progression. BNP levels are very high in the patients with HF, but remain low in the patients with acute dyspnea due to other causes such as chronic obstructive pulmonary disease, asthma or obesity. Plasma BNP values increase with increasing age and are higher in women than in men (2).

Unstable angina, for example is a common transitory phase of coronary ischemia, bordering on myocardial infarction (MI). It is a strong relationship with BNP and outcomes in ACS patients (8).

It has been previously reported that 21% of ambulatory patients with established chronic heart failure who are stable may have plasma BNP levels less than 100 pg/mL. All commercially available BNP assays incorporate the value 100 pg/mL as the diagnostic cut off (9). If BNP level is 100-500 pg/mL that requires further diagnostic evaluation ("grey zone"). If BNP is higher than 500 pg/mL there is probability of the hearth failure (10).

Troponins I, T and C are structural proteins bound to the thin filaments (actin) in striated muscle. A small amount (5-8%) of troponin exists free in the cytosol. Elevated levels of cTnI (above the values established for non-MI specimens) are detectable in serum within 4 to 6 hours after the onset of chest pain, reach peak concentration in approximately after 8 to 28 hours, and remain elevated for 3 to 10 days following MI. Cardiac troponin is the preferred biomarker for the

detection of myocardial injury based on improved sensitivity and superior tissue-specificity compared to other available biomarkers of necrosis, including CK-MB, myoglobin, lactate dehydrogenase, and others. The high specificity of cTnI measurements is beneficial in identify cardiac injury for clinical conditions involving skeletal muscle injury resulting from surgery, trauma or muscular disease (11). The Joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation Task Force redefinition of acute myocardial infarction (AMI) is predicated on the detection of increase or decrease of cardiac troponin (cTn), with at least 1 concentration above the 99th presence reference value in patients with evidence of myocardial ischemia. Blood samples for measurement of cTn are recommended to be drawn at presentation and 6-9 h later to optimize clinical sensitivity for ruling in AMI (12,13). The reference range for troponin I (TnI) in serum is 0.00-0.032 µg/mL.

In our study we have measured BNP and TnI blood levels in the patients with ACS in a first 12 hours and investigate correlation with peak value of TnI.

METHODS

Patients

Our research included patients (n = 100) and 50 healthy control group in period from January till September 2011. The retrospective study included patients who were hospitalized at the Heart Disease Department at the University Clinical Center Sarajevo. In our study we included patients with acute myocardial infarction (AMI) and unstable angina. The clinical spectrum of ACS consists of ST elevated myocardial infarction (STEMI) and non-ST elevated myocardial infarction (NSTEMI)/or unstable angina (UA), which are classified using electrocardiography (ECG) changes. The study included patients who had a level of BNP more than 100 pg/mL and level of TnI more than 0.032 µg/mL. Our research included determination of BNP and TnI in blood of patients in a first 12 hours of ACS symptoms.

The healthy control group included patients without AMI and unstable angina using electrocardiography (ECG), BNP level < 100 pg/mL and TnI level <0.032 µg/mL. The patients with history of

pulmonary thromboembolism, acute and chronic renal failure, end stage renal disease, sepsis, liver cirrhosis, chronic obstructive lung disease, hyperthyroidism and adult respiratory distress syndrome were excluded from the study. The research was done respecting ethical standards in the Helsinki Declaration.

Specimen preparation

Na-EDTA plasma should be used for the Architect BNP assay. Samples should be collected in plastic collection tubes, because the BNP molecule has proven to be unstable in glass containers. Specimens containing blood cells or particle matters may give inconsistent results and must be clarified by centrifugation prior to testing. Specimens with BNP assay value exceeding 5000.0 pg/mL are flagged with the code ">5000.0 pg/mL" and may be diluted using the Automated Dilution Protocol. The samples for determination of TnI should be collected in the tubes with gel. The TnI assay concentration greater than 50 ng/mL may be diluted using the Automated Dilution Protocol. The patients' samples of blood were collected in Na-EDTA and gel Vacutainer test tubes (Becton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL.

Assays

All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody. A label is a molecule that will react as a part of the assay, so that a change in signal can be measured in the blood after added reagent solution. CMIA is a noncompetitive sandwich assay technology to measure analytes. The amount of signal is directly proportional to the amount of analyte present in the sample.

Chemiluminescent microparticle immunoassay – CMIA

Architect BNP or TnI assay is a two-step immunoassay to determine the presence of BNP and TnI in human blood using CMIA technology. As a first step, sample, assay diluent and anti-antibody-coated paramagnetic particles are combined. BNP or TnI present in the sample binds to the anti-coated microparticles. After incubation and wash, anti-acridinium-labeled conjugate is added in the second step.

Following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The pre-trigger solution (hydrogen peroxide) creates an acidic environment to prevent early release of energy (light emission), helps to keep microparticles from clumping and splits acridinium dye off the conjugate bound to the microparticle complex (this action prepares the acridinium dye for the next step). The trigger solution (sodium hydroxide) dispenses to the reaction mixture. The acridinium undergoes an oxidative reaction when it is exposed to peroxide and an alkaline solution. This reaction causes the occurrence of chemiluminescent reaction. N-methylacridone forms and releases energy (light emission) as it returns to its ground state. The resulting chemiluminescent reaction is measured as relative light units (RLU). A direct relationship exists between the amount of BNP in the sample and RLU detected by Architect System optics. The concentration of BNP or TnI will be read relative to a standard curve established with calibrators of known BNP and TnI concentration.

Statistical analysis

The results were statistically analyzed using NCSS and statistical software SPSS version 12.0 software, determined by the average value (\bar{x}), standard deviation (SD) or median and interval. The data were not distributed normally we use Mann Whitney U-test. Pearson correlation test was used to assess association between measured parameters. P – Values less than <0.05 was considered as statistically significant.

RESULTS

The serum concentrations of BNP and TnI in the patients with AMI (acute myocardial infarction) and unstable angina are shown in Table 1. The study included 100 patients (53 men and 57 women), they were classified depending on their diagnosis and healthy control group without ACS. The average age was 64 years for the AMI patients, and 61 years for the patients with unstable angina. The value of BNP and TnI was higher in the group with AMI than the group with unstable angina. The healthy control group had a lower concentration of BNP and TnI than the patient groups.

Using Mann Whitney U test we made comparison of BNP and TnI levels among the groups including the

TABLE 1. The mean concentration of biochemical parameters in groups with AMI, unstable angina and healthy control

	AMI group	Unstable angina group	Healthy control group	P value
BNP (pg/mL)	764.48	287.18	18.74	p <0.001
SD	639.52	593.2	7.64	
SEM	90.44	83.44	1.08	
Median	585.25	98.1	19.1	
Interval	260-4441	18-2514	10-33.10	
TnI (ng/mL)	2.5	0.1	0.01	p <0.001
SD	2.28	0.23	0.024	
SEM	0.32	0.0033	0.0034	
Median	2.19	0.02	0	
Interval	0.31-7.07	0.00-1.10	0.00-0.09	

patients with AMI, unstable angina and the healthy control group. According to Mann-Whitney U test for $\alpha = 5\%$ the difference between concentrations of BNP in the patients with AMI and the patients with unstable angina were significant. The same test for $\alpha = 5\%$ has shown a significant difference between concentrations of BNP in patients with AMI and healthy control group. Using Mann Whitney U test we made comparison between serum TnI concentration in the group of AMI patients and the healthy control group. The results between the groups were statistically significant for $P < 0.05$. The same test has shown a significant difference between concentrations of TnI in the patients with AMI and the patients with unstable angina for $P < 0.05$.

In our study we found a significant correlation between the average concentrations of TnI and BNP with Pearson correlation coefficient ($r = 0.36$). Regression equation revealed a slope of 344.09 and a y axis intercept of 457.83. The results between average concentrations of TnI and BNP were statistically significant for $P < 0.05$ using Student t test, the results are shown in Figure 1.

DISCUSSION

Natriuretic peptides elevations have shown the correlation with wall stress, and thus provided functional information. The level of plasma BNP depends on the equilibrium between myocardial secretion as compensatory response to injury or wall stress and an amount and activity of expressed guanylyl cyclase-type BNP receptors and also peripheral degradation rate of BNP through neutral endopeptidases. The

ischemia induced by increase in ventricular wall stress that induced release of BNP. The TnI elevations are seen in multiple chronic cardiac and noncardiac conditions, a rise or fall in serial measurement of TnI levels strongly supports an acutely evolving cardiac injury such as, most commonly, acute myocardial infarction (14). In our study we found significant elevated levels of plasma BNP and TnI in acute myocardial infarction. In our study we determined the value of BNP 764.48 ± 639.52 pg/mL ($260-4441$ pg/mL) in the patients with AMI. The level of TnI in the group with AMI was 2.50 ± 2.28 ng/mL ($0.31-7.07$ ng/mL). Grybauskiene R. and al. (15) have got the mean concentration of TnI 0.499 ng/mL ($0.07-2.89$ ng/mL) and BNP level 758 pg/mL ($206-2158$ pg/mL). In our study patients with unstable angina had the concentration of BNP 287.18 ± 593.20 pg/mL ($18-2514$ pg/mL) and TnI level 0.10 ± 0.23 ng/mL ($0.00-1.10$ ng/mL) and healthy control group has concentration of BNP 18.74 ± 7.64 pg/mL ($10-33.10$ pg/mL) and TnI level 0.01 ± 0.024 ng/mL ($0.00-0.09$ ng/mL). It is a lower concentration of BNP and TnI than in the patients with AMI, the results are shown in Table 1. The other researchers have got results of BNP 70.2 ± 53.3 pg/mL in the patients with unstable angina (16). In the present study, we have shown significantly higher BNP plasma level by patients with AMI in compare BNP level in healthy group results are shown in Table 1. The similarly results have got Morita and al. (17) and Richards and al. (18). Patients with elevated plasma BNP levels (>80 pg/mL) had a significantly higher incidence of new heart failure and all-cause

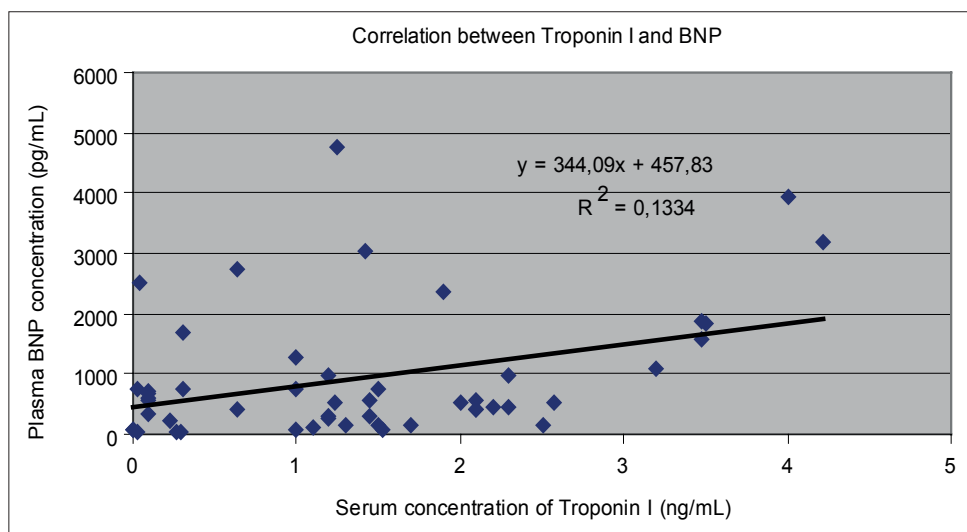


FIGURE 1. Comparison of TnI and BNP in blood measured by Architect CMIA correlation coefficient $r = 0.36$.

mortality than those with a normal plasma BNP level (≤ 80 pg/mL) (19). In our study, patients with BNP level 80 pg/mL have stayed longer in the Department of Heart Diseases and had a higher incidence of new heart failure. The data of While HD and al. (14) have shown that BNP concentration is increased during AMI and occurring after the first AMI. BNP concentration in plasma during AMI is strongly related to the marker of myocardial necrosis reflecting the extent of injured myocardium, and to degree of acute heart failure. During AMI BNP levels correlated strongly with TnI. In our study we have got good correlation of BNP and TnI in patients with AMI. In correlation between BNP and troponin we got correlation coefficient $r = 0.36$ with statistical significance for $p < 0.05$. The results are shown in Figure 1. The other researchers have got results of BNP and troponin correlation with correlation coefficient $r = 0.273-0.70$ (15, 19). Necrosis and apoptosis of myocytes in AMI are contributions of progressive left ventricle dysfunction. Therefore we have done a correlation between BNP and TnI to contribute that BNP as TnI could be a marker of myocytes necrosis in patients with AMI. The results of Karcaiauskaite have shown a correlation coefficient $r = 0.72$ indicating strongly correlation between BNP and TnI. The reason why we got lower correlation coefficient is a fact that BNP gene transcription is increased both in infarcted tissue

and it surrounding ischemic but viable myocardium whose extent differs (20). Studies have shown that BNP secretion and BNP mRNA expression are increased mainly in the borderline region between the infarcted and non-infarcted regions. The stimulus for this appears to be increased all stress directly related to the infarction. The clinical ischemia is result of extensive necrosis is associated with release of BNP. Ischemia itself rather than changes in wall stress secondary to ischemia might promote BNP release (21,22). Our study show that BNP can predict high risk features in ACS such as more severe underlying atherosclerosis, left ventricular hypertrophy and burden of ischemic insult. The patients with higher BNP have worse prognosis of AMI even with normal value of TnI. Therefore BNP could be used as a marker of myocardial necrosis as well as marker of risk for myocardium ischemic viable.

CONCLUSION

In our study BNP plasma levels are significant higher in AMI in compared with unstable angina group and healthy control group. Plasma level BNP was elevated in patients with left ventricular (LV) dysfunction. Serial measurements of plasma BNP and TnI concentrations might be a useful tool for identification of patients at risk of developing AMI and unstable angina. In patients with ACS BNP

adds important prognostic information to clinical and laboratory variables as well as levels of troponin. Determination of BNP rise could be used for quick and easy estimation of infarction size. BNP together with TnI levels in acute phase of myocardial infarction might be useful in predicting subsequent cardiac function.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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