

Acute Changes in Circulating Natriuretic Peptide Levels in Relation to Myocardial Ischemia

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OBJECTIVES	The aim of this study was to determine the effect of transient myocardial ischemia on circulating natriuretic peptide levels.
BACKGROUND	Natriuretic peptides are released by the heart in response to wall stress. We hypothesized that transient myocardial ischemia would cause acute changes in circulating natriuretic peptide levels.
METHODS	B-type natriuretic peptide (BNP), N-terminal fragment of BNP pro-hormone (NT-pro-BNP), and N-terminal fragment of atrial natriuretic peptide pro-hormone (NT-pro-ANP) levels were measured in 112 patients before, immediately after, and 4 h after exercise testing with nuclear perfusion imaging.
RESULTS	Baseline levels of BNP were associated with the subsequent severity of provoked ischemia, with median levels of 43, 62, and 101 pg/ml in patients with none, mild-to-moderate, and severe inducible ischemia, respectively ($p = 0.03$). Immediately after exercise, the median increase in BNP was 14.2 pg/ml in patients with mild-to-moderate ischemia ($p = 0.0005$) and 23.7 pg/ml in those with severe ischemia ($p = 0.017$). In contrast, BNP levels only rose by 2.3 pg/ml in those who did not develop ischemia ($p = 0.31$). A similar relationship was seen between baseline NT-pro-BNP levels and inducible ischemia, but the changes in response to ischemia were less pronounced. NT-pro-ANP levels rose with exercise in both ischemic and non-ischemic patients. When added to traditional clinical predictors of ischemia, a post-stress test BNP ≥ 80 pg/ml remained a strong and independent predictor of inducible myocardial ischemia (odds ratio 3.0, $p = 0.025$).
CONCLUSIONS	Transient myocardial ischemia was associated with an immediate rise in circulating BNP levels, and the magnitude of rise was proportional to the severity of ischemia. These findings demonstrate an important link between the severity of an acute ischemic insult and the circulating levels of BNP. (J Am Coll Cardiol 2004;44:1988–95) © 2004 by the American College of Cardiology Foundation

Natriuretic peptides are vasoactive hormones secreted by the heart. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are synthesized as pro-hormones and cleaved by peptidases into the C-terminal active hormones and the N-terminal pro-hormone fragments (1). BNP in particular has been shown to be a powerful diagnostic and prognostic marker in heart failure (2,3). More recently, BNP has been studied in acute coronary syndromes. In ST-segment elevation myocardial infarction (MI), BNP rises substantially and rapidly, with elevated levels apparent on presentation and peak concentrations occurring by 16 h

(4). Elevated levels of BNP can also be detected in a subset of patients presenting with non-ST-segment elevation acute coronary syndromes (5), even in those without biochemical evidence of myocardial necrosis or clinical evidence of heart failure (6).

Given that the primary stimulus for natriuretic peptide secretion is increased myocardial wall stress, circulating levels of these short-lived hormones might provide insight into transient changes in ventricular systolic and diastolic function during acute myocardial ischemia, which can impair both myocardial relaxation and contractility. Therefore, we hypothesized that small but demonstrable amounts of natriuretic peptides would be released in response to transient myocardial ischemia. We tested this hypothesis by examining circulating BNP, N-terminal fragment of BNP pro-hormone (NT-pro-BNP), and N-terminal fragment of ANP pro-hormone (NT-pro-ANP) levels before and after exercise stress testing and correlating the results with scintigraphic evidence of inducible ischemia.

METHODS

Patients. A total of 155 patients who were undergoing stress testing with myocardial perfusion imaging at Brigham and Women's Hospital were enrolled in PROtein Markers

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Abbreviations and Acronyms

ANP	= atrial natriuretic peptide
BNP	= B-type natriuretic peptide
CABG	= coronary artery bypass graft
CAD	= coronary artery disease
CHF	= congestive heart failure
CI	= confidence interval
LV	= left ventricular
MI	= myocardial infarction
NT-pro-ANP	= N-terminal fragment of ANP pro-hormone
NT-pro-BNP	= N-terminal fragment of BNP pro-hormone
OR	= odds ratio

of ischemia using Proteomic Testing (PROMPT)-Thrombolysis In Myocardial Infarction (TIMI) 35, a prospective cohort study. The Human Research Committee approved the study protocol, and all patients provided written informed consent. All patients who were referred for stress testing for the evaluation of possible myocardial ischemia were eligible for participation. Patients who underwent pharmacologic or submaximal exercise stress testing ($n = 41$) or those in whom adequate perfusion images were not obtained ($n = 2$) were excluded, leaving a total of 112 patients included in these analyses.

Study protocol. Data were obtained on each patient's age, gender, race, weight, cardiac risk factors (including hypertension, diabetes mellitus, smoking, and hyperlipidemia), previous cardiac disease (including angina, MI, congestive heart failure [CHF], angiographically confirmed significant coronary artery disease [CAD], percutaneous coronary intervention, and coronary artery bypass grafting [CABG]), and cardiac medications.

Patients underwent exercise testing using the standard Bruce protocol. Duration of the stress test, metabolic equivalents achieved, peak heart rate, and peak blood pressure were recorded. If the patient developed angina during the test, the timing, quality (typical vs. atypical), and effect on the test (limiting or non-limiting) were noted. The maximal horizontal or downsloping ST-segment changes were recorded in each electrocardiogram lead.

Single-photon emission computed tomography myocardial perfusion imaging. ^{99m}Tc tetrofosmin was administered at peak stress, and imaging was performed soon thereafter. Four hours later, a second injection was administered and repeat imaging was performed. A 20-segment myocardial model was used for semiquantitative analysis, with a visual perfusion rating of 0 to 4 for each segment by nuclear cardiologists unaware of biomarker data. Quantitative analysis of perfusion was also performed using the CEQUAL method to calculate the percent reversible and fixed perfusion defects. Patients were categorized as having none (no reversible perfusion defects), mild-to-moderate (change in visual scale score of 1 to 10 points), or severe ischemia

(change in visual scale score of >10 points or a change of >5 points and a reversible defect percentage of $\geq 10\%$).

Blood samples were obtained immediately before, immediately after (median 7 min), and 4 h after stress testing. Blood samples were placed on ice and processed within 60 min. Plasma was stored at -80°C , and aliquots were thawed for these analyses.

Biomarkers. An established sequential sandwich immunoassay (Biosite, Inc., San Diego, California) was used for the quantification of BNP (5,6). The minimal detectable concentration is 4 pg/ml. The coefficient of variation is 5.0% at 53.8 pg/ml. NT-pro-BNP was measured on the Elecsys 2010 (Roche Diagnostics, Basel, Switzerland). The minimal detectable concentration is 50 pg/ml, and the coefficient of variation is 5.7% at 64 pg/ml. NT-pro-ANP was measured using a competitive-binding radioimmunoassay (7). The minimal detectable concentration is 100 pmol/l, and the coefficient of variation is 7.5% at 425 pmol/l. All assays were performed by personnel who had no knowledge of stress test results.

Statistical analyses. Patients were categorized for the primary analysis on the basis of the severity of ischemia as determined by myocardial perfusion imaging. Median natriuretic peptide levels at each time point were compared across groups using a non-parametric test for trend across ordered groups (Jonckheere-Terpstra test) (8). The median differences between baseline and post-stress test natriuretic peptide levels were compared using signed rank tests. To adjust for differences in baseline factors, linear regression was used after confirming that the residuals in such models were approximately normally distributed. Covariates in the model included age; gender; a history of hypertension; previous MI, CABG, or CHF; use of beta-blockers, nitrates, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or digoxin; exercise duration and percent of maximal predicted heart rate that was achieved; and low ejection fraction. BNP was also considered as a categorical variable using a prespecified threshold of 80 pg/ml (based on previous work) (5,6). The independent value of BNP for predicting ischemia was calculated using a multivariable logistic regression model that contained BNP ≥ 80 pg/ml plus additional covariates from the Duke treadmill score including exercise time, the presence of angina (non-limiting or limiting), and the magnitude of ST-segment depression (0, 1, or 2 mm), as well as using a model that contained the aforementioned covariates plus age, gender, cardiac risk factors (hypertension, diabetes, hypercholesterolemia, and smoking), and previous cardiac disease and procedures (angina, documented significant coronary artery stenosis, previous MI, percutaneous coronary intervention, CABG, and CHF).

RESULTS

Baseline characteristics. The baseline characteristics of the 112 study participants are shown in Table 1. The mean

Table 1. Patient Characteristics (n = 112)

Demographics	
Age (yrs)	63 ± 11
Male	84 (75%)
White	87 (78%)
Cardiac risk factors	
Hypertension	83 (74%)
Diabetes	27 (24%)
Current or former smoker	73 (65%)
Hyperlipidemia	83 (74%)
Cardiac disease	
Documented CAD	63 (57%)
Previous MI	55 (49%)
Previous PCI	40 (36%)
Previous CABG	26 (23%)
Previous CHF	16 (14%)
Cardiac medications	
Aspirin	93 (83%)
Beta-blocker	94 (84%)
Nitrates	23 (21%)
Calcium channel blocker	25 (22%)
ACE inhibitor	41 (37%)
Angiotensin receptor blocker	5 (4%)
Hypolipidemic	79 (71%)
Diuretic	33 (29%)
Digoxin	7 (6%)

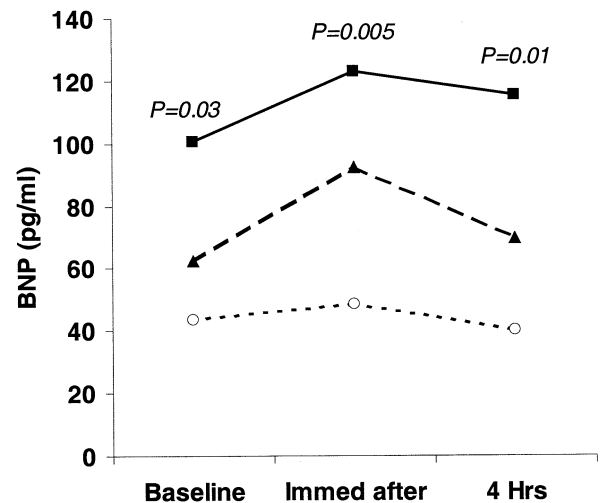
Data are presented as mean values ± SD or number (%) of patients.

ACE = angiotensin-converting enzyme; CABG = coronary artery bypass graft; CAD = coronary artery disease; CHF = congestive heart failure; MI = myocardial infarction; PCI = percutaneous coronary intervention.

age was 63 years. Seventy-five percent of patients were male, 74% had hypertension, 57% had known CAD, 49% had a previous MI, 36% had undergone a percutaneous coronary intervention, 23% had undergone CABG, and 14% had a history of heart failure. The majority of patients were taking aspirin, beta-blockers, and hypolipidemics. One-quarter to one-third of patients were taking nitrates, calcium channel blockers, angiotensin-converting enzyme inhibitors, and diuretics.

Exercise testing. Mean exercise duration was 7.4 ± 2.9 min, maximum workload achieved was 8.7 ± 3.1 metabolic equivalents, and percent of maximum predicted heart rate was 81 ± 12%. Twenty-two percent of patients had non-limiting angina and 9% had limiting angina. Horizontal or downsloping ST depression ≥1 mm developed in 30% of patients and ≥2 mm in 15% of patients. Forty percent of patients had no inducible ischemia, 46% had mild-to-moderate inducible ischemia, and 13% had severe ischemia. The mean left ventricular (LV) ejection fraction was 55 ± 13%. Twenty-eight percent of patients had a LV ejection fraction <50%.

Natriuretic peptide levels before and after stress testing. Circulating plasma BNP levels immediately before, immediately after, and 4 h after stress testing are shown in Figure 1. In patients without demonstrable inducible ischemia, median BNP levels were low at baseline (43 pg/ml) and were not significantly changed immediately after stress testing (49 pg/ml) or at 4 h (40 pg/ml). In contrast, BNP levels rose significantly immediately after exercise in patients with inducible ischemia. Among patients with mild-to-



Ischemia	n	Median BNP (IQR) pg/ml
Severe	14	101 (67-151) / 123 (64-263) / 115 (54-183)
Mild/Mod	42	62 (43-104) / 92 (54-141) / 70 (35-97)
No	40	43 (29-114) / 49 (22-125) / 40 (21-90)

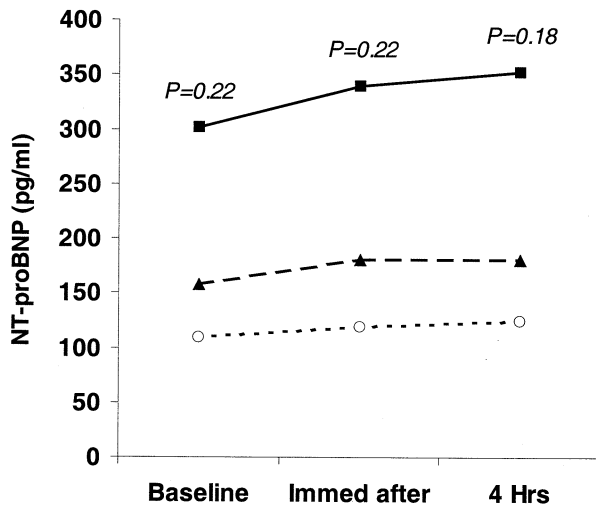
Figure 1. Median B-type natriuretic peptide (BNP) levels in patients with no (open circles), mild-to-moderate (black triangles), and severe (black squares) ischemia, in whom samples were available at all three time points (baseline, immediately [Immed] after stress testing, and 4 h after stress testing). The p values are for trend across ischemic categories at each time point. IQR = interquartile range; Mod = moderate.

moderate ischemia, median BNP levels rose from 62 to 92 pg/ml (p = 0.0005). By 4 h, levels had returned close to baseline (70 pg/ml). Among patients with severe ischemia, median BNP levels were high at baseline at 101 pg/ml and rose even higher after stress testing to 123 pg/ml (p = 0.017). By 4 h after stress testing, BNP levels remained elevated at 115 pg/ml but were trending toward baseline levels.

Levels of NT-pro-BNP are shown in Figure 2. In patients without demonstrable inducible ischemia, median NT-pro-BNP levels were low at baseline and did not change significantly immediately after stress testing or at 4 h (109, 119, and 124 pg/ml, respectively). In patients with mild-to-moderate ischemia, median NT-pro-BNP levels were 158 pg/ml at baseline, exhibited a mild increase to 181 pg/ml immediately after stress testing (p < 0.01), and remained at 181 pg/ml at 4 h. In patients with severe ischemia, median NT-pro-BNP levels were 302 pg/ml at baseline, rose to 340 pg/ml immediately after stress testing (p = 0.13), and reached 352 pg/ml at 4 h.

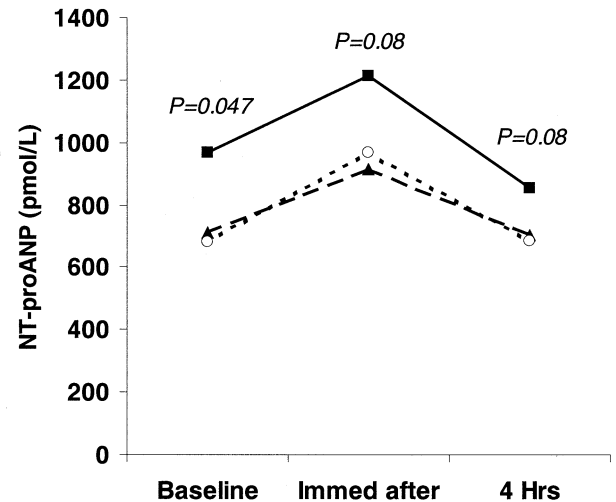
Levels of NT-pro-ANP are shown in Figure 3. Even in patients without demonstrable inducible ischemia, median NT-pro-ANP levels rose from 680 pmol/l at baseline to 969 pmol/l immediately after stress testing (p = 0.0001), and then fell back down to 686 pmol/l by 4 h. Similarly, in patients with mild-to-moderate ischemia, median NT-pro-ANP levels were 710 pmol/l at baseline, rose to 914 pmol/l immediately after stress testing (p = 0.0001), and then fell to 703 pmol/l at 4 h. In patients with severe ischemia,

Acute Changes in BNP With Myocardial Ischemia



Ischemia	n	Median NT-proBNP (IQR) pg/ml		
Severe	12	302 (41-1497)	340 (45-1742)	352 (66-1576)
Mild/Mod	38	158 (101-316)	181 (113-384)	181 (119-409)
No	29	109 (42-469)	119 (47-546)	124 (43-572)

Figure 2. Median N-terminal fragment of BNP pro-hormone (NT-pro-BNP) levels in patients with no (open circles), mild-to-moderate (black triangles), and severe (black squares) ischemia, in whom samples were available at all three time points (baseline, immediately [Immed] after stress testing, and 4 h after stress testing). The p values are for trend across ischemic categories at each time point. IQR = interquartile range; Mod = moderate.



Ischemia	n	Median NT-proANP (IQR) pmol/L		
Severe	12	967 (667-1885)	1215 (779-2450)	854 (644-2090)
Mild/Mod	38	710 (572-1240)	914 (763-1480)	703 (587-1180)
No	30	680 (466-1120)	969 (581-1380)	686 (450-1250)

Figure 3. Median N-terminal fragment of ANP pro-hormone (NT-pro-ANP) levels in patients with no (open circles), mild-to-moderate (black triangles), and severe (black squares) ischemia, in whom samples were available at all three time points (baseline, immediately [Immed] after stress testing, and 4 h after stress testing). The p values are for trend across ischemic categories at each time point. IQR = interquartile range; Mod = moderate.

median NT-pro-ANP levels were 967 pmol/l at baseline, rose to 1,215 pmol/l immediately after stress testing ($p = 0.009$), and then fell to 854 pmol/l at 4 h.

Thus, the circulating levels of the three different natriuretic peptides displayed different patterns in response to exercise with and without ischemia. BNP levels differed across the ischemic categories at all three time points, and there was a robust rise (~25% of baseline levels) in ischemic but, importantly, not in non-ischemic patients. In contrast, NT-pro-BNP levels did not differ statistically across ischemic categories and the rise in ischemic patients was more modest (<10% of baseline levels). For NT-pro-ANP, a rise was seen with exercise for all patients and did not differ statistically across ischemic categories (130 vs. 150 vs. 234 pmol/l; $p = 0.16$). Thus, the remainder of the analyses focuses on BNP levels.

Gradient of BNP levels at baseline and after stress testing. The differences in BNP levels at baseline (43 vs. 62 vs. 101 pg/ml in patients with none, mild-to-moderate, and severe inducible ischemia, respectively) were statistically significant ($p = 0.03$). Moreover, this gradient remained apparent after excluding patients with either a LV ejection fraction <50% or a clinical history of heart failure: 43 versus 56 versus 97 pg/ml ($p = 0.08$). Using a cut point of 80 pg/ml, 30%, 38%, and 64% of patients with none, mild-to-moderate, and severe inducible ischemia, respectively, had elevated levels ($p = 0.036$). Again, this trend remained apparent after excluding patients with LV dysfunction or a clinical history of heart failure: 26%, 31%, and 56% ($p =$

0.14). In a multivariable logistic model, inducible ischemia was more frequent among patients with an elevated baseline BNP compared with those with a baseline BNP <80 pg/ml (adjusted odds ratio [OR] 2.1, 95% confidence interval [CI] 0.9 to 5.0). Excluding patients with LV dysfunction or a clinical history of heart failure, the adjusted OR was 1.7 (95% CI 0.6 to 4.9).

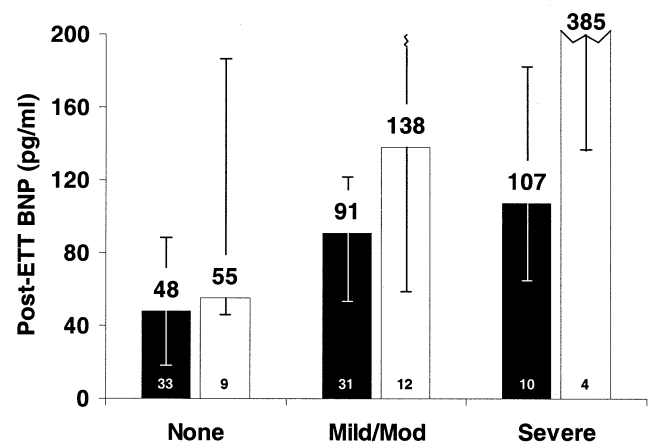


Figure 4. Median post-stress test B-type natriuretic peptide (BNP) levels with interquartile ranges in patients with none, mild-to-moderate, and severe ischemia, stratified into those with ejection fractions $\geq 50\%$ (black bars) and $< 50\%$ (white bars). Numbers within each bar represent the number of patients in that group. The p values are for trend across ischemic categories ($p = 0.14$ for patients with ejection fraction < 0.5 ; $p = 0.012$ for patients with ejection fraction ≥ 0.5). ETT = exercise tolerance test; Mod = moderate.

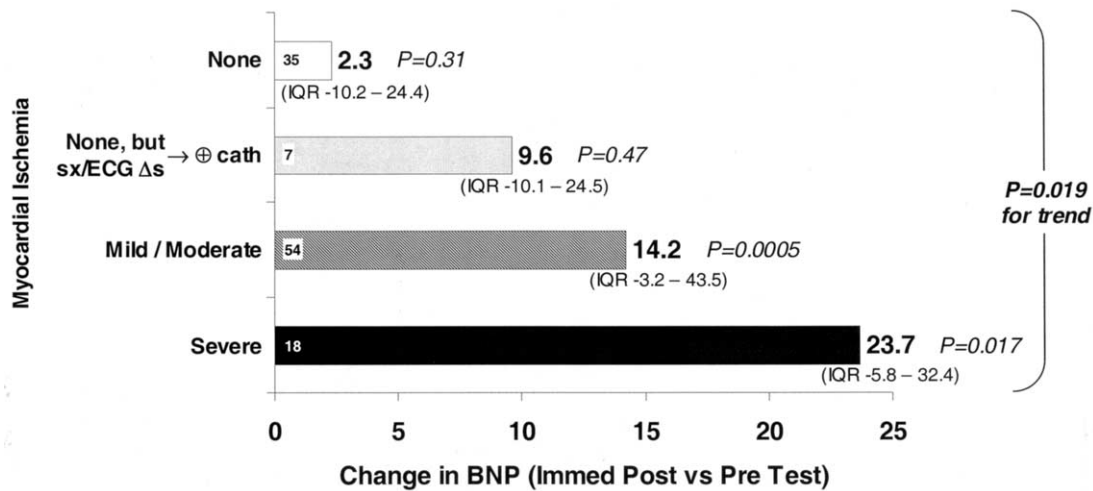


Figure 5. Median changes in B-type natriuretic peptide (BNP) levels in response to exercise stress testing in patients with no ischemia (white bar), with no ischemia on scintigraphy but subsequently shown to have critical coronary disease (light gray bar), with mild-to-moderate ischemia (dark gray bar), and with severe ischemia (black bar). Numbers within each bar represent the number of patients in that group. The p values by each bar are for the median change. The p value at the far right is for the trend across ischemic categories. cath = cardiac catheterization; ECG = electrocardiographic; Immed = immediately; IQR = interquartile range; sx = symptoms.

The differences in circulating BNP levels between groups became more pronounced after stress testing. Immediately after exercise, BNP levels were 49, 92, and 123 pg/ml, respectively, in patients with none, mild-to-moderate, and severe inducible ischemia ($p = 0.005$). This gradient was apparent both in patients with preserved LV ejection fractions and in those with reduced LV ejection fractions (Fig. 4). Using a cut point of 80 pg/ml, 31%, 56%, and 71% of patients with none, mild-to-moderate, and severe inducible ischemia, respectively, had elevated levels ($p = 0.003$). This trend remained significant after excluding patients with impaired LV ejection fraction or a clinical history of heart failure: 27%, 53%, and 67% ($p = 0.01$).

Changes in circulating BNP levels. The median rise in BNP levels in response to exercise stress testing in patients categorized by myocardial ischemia is shown in Figure 5. Of note, seven patients had no evidence for inducible ischemia on perfusion imaging, but had symptoms or electrocardiographic changes sufficiently compelling as to lead to cardiac catheterization. All of these patients were found to have critical coronary artery stenoses at coronary angiography and are shown separately. Interestingly, the median increase in patients with normal perfusion imaging but significant coronary disease (9.6 pg/ml) was intermediate between the increase seen in patients with mild-to-moderate ischemia on perfusion imaging (14.2 pg/ml) and the change seen in patients without ischemia (2.3 pg/ml).

After adjusting for differences in baseline characteristics (including age; gender; a history of hypertension, MI, CABG, or CHF; use of beta-blockers, nitrates, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or digoxin; exercise duration and percent of maximal predicted heart rate that was achieved; and low ejection fraction), mild-to-moderate ischemia was associated with a

18.5 pg/ml rise in BNP ($p = 0.021$) and severe ischemia was associated with a 28.5 pg/ml rise in BNP ($p = 0.015$).

We explored the relationship between age and beta-blocker use and the rise of BNP in response to ischemia. Patients older than the median age (63 years) appeared to manifest greater rises in BNP in response to ischemia (an additional 10.8 pg/ml, 95% CI -17.7 to 39.3 pg/ml) as did patients taking beta-blockers (an additional 13.0 pg/ml, 95% CI -25.6 to 51.7 pg/ml), although neither interaction reached statistical significance.

BNP to predict ischemia. We created a multivariable logistic regression model for inducible ischemia in which the predictor variables were the components of the Duke treadmill score (i.e., duration of exercise, presence and type of angina, and magnitude of ST-segment depression) plus post-stress test BNP as a categorical variable using a cutoff of 80 pg/ml. As can be seen in Figure 6, an elevated BNP after stress testing was a strong and independent predictor of ischemia, with an adjusted OR of 3.03 (95% CI 1.15 to 8.02, $p = 0.025$). Reclassifying as ischemic those patients with no reversible defects on imaging but who were ultimately found to have significant CAD revealed a more pronounced predictive ability of BNP, with an adjusted OR of 4.4 (95% CI 1.5 to 13.0, $p = 0.008$). The inclusion of additional covariates including age, gender, cardiac risk factors (hypertension, diabetes, hypercholesterolemia, and smoking), and previous cardiac disease and procedures (angina, documented significant coronary artery stenosis, previous MI, percutaneous coronary intervention, CABG, and CHF) did not change the predictive power of an elevated BNP (OR 3.8, 95% CI 1.1 to 14.0, $p = 0.04$). Restricting the analyses to patients with preserved LV systolic function, an elevated BNP after stress testing was an even stronger predictor of inducible ischemia with an adjusted OR of 5.9 (95% CI 1.2 to 28.9, $p = 0.03$).

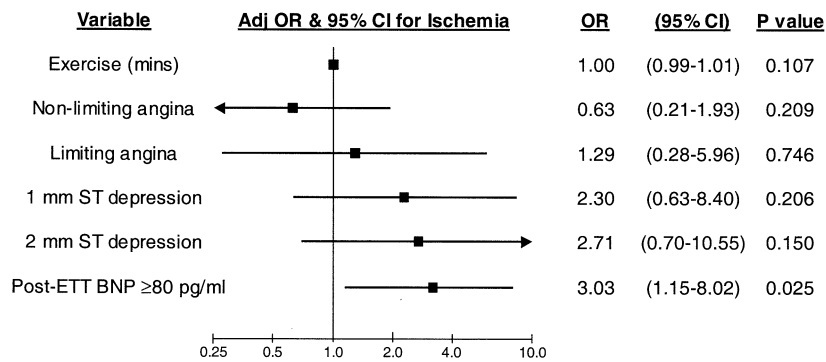


Figure 6. Point estimates and 95% confidence intervals (CI) for the odds ratio (OR) for the components of the Duke Treadmill Score and a post-stress test B-type natriuretic peptide (BNP) ≥ 80 pg/ml. Adj = adjusted; ETT = exercise tolerance test.

An elevated post-stress test BNP, at a cut point of 80 pg/ml, had a sensitivity of 60%, a specificity of 69%, and an accuracy of 64%. Similar results were obtained when using the rise in BNP at a cut point of 10 pg/ml: the sensitivity was 60%, the specificity was 69%, and the accuracy was 64%. Whereas the traditional predictors of myocardial ischemia on stress testing, including male gender and all of the components of the Duke treadmill score combined, had only moderate discriminatory capacity ($c = 0.74$), by combining the traditional factors and an elevated post-stress test BNP, discriminatory capacity was improved ($c = 0.82$). When patients were categorized on the basis of how many of the four risk factors were present (male gender, limiting angina, ST-segment depression, post-stress test BNP ≥ 80 pg/ml), a stepwise and significant gradient of risk for inducible myocardial ischemia was observed, ranging from 25% to 100% (Fig. 7, $p < 0.001$).

DISCUSSION

Initial studies demonstrated the ability of BNP levels to predict death and adverse cardiac events in the setting of acute ST-segment elevation MI (3). We subsequently extended those observations across the spectrum of acute coronary syndromes using samples obtained a median of 40 h from the onset of symptoms (5). In a later study, we demonstrated the prognostic utility of BNP levels in samples obtained within 24 h of presentation (6). We now provide evidence for an even more immediate and quantitative link between acute transient myocardial ischemia and the plasma concentration of BNP.

Our study has three major findings. First, patients with greater ischemic burdens had higher baseline resting levels of BNP, with qualitatively similar patterns for NT-pro-BNP and NT-pro-ANP. Second, circulating BNP levels rose in response to exercise stress testing, and the magnitude of that rise was proportional to the degree of inducible ischemia. The increase, however, was less pronounced for NT-pro-BNP and was not as specific for ischemia in regard to NT-pro-ANP. Third, an elevated concentration of BNP (≥ 80 pg/ml) after exercise stress testing was a significant predictor of inducible ischemia, comparable to and inde-

pendent of traditional parameters such as limiting angina and ST-segment depression. However, neither the absolute value nor the magnitude of rise of BNP post-stress testing was sufficiently sensitive or specific by itself for the diagnosis of inducible ischemia.

Whereas ANP is stored as pro-ANP and cleaved upon release into ANP and NT-pro-ANP, immunochromatography studies suggest that the primary form of BNP stored in the heart is the C-terminal BNP itself (9). This might explain why the absolute acute increases seen for BNP were greater than that for NT-pro-BNP, an observation we have also made in the setting of heart failure (10). In addition, the longer half-life of NT-pro-BNP compared with BNP (2 to 3 h vs. 18 min) leads to higher baseline circulating levels of NT-pro-BNP. Therefore, small absolute increases in NT-pro-BNP may be harder to detect. The longer half-life of NT-pro-BNP may also explain why the acute increases seen in NT-pro-BNP appeared to be more persistent. With regard to NT-pro-ANP, although the acute changes we observed with exercise were robust, they were not as specific for the presence of inducible ischemia. This may reflect the fact that ANP is released in response to tachycardia alone (11). This non-specific increase in circulating levels of ANP in the setting of exercise may therefore mask all but the most severe changes resulting from ischemia (12).

In terms of BNP, several studies show that it appears to be released in response to increased wall stress (13,14). Ischemia can cause transient LV systolic and diastolic dysfunction. We therefore speculate that individuals who develop ischemia during exercise also develop increases in LV end-diastolic wall tension, thereby triggering the release of BNP from granules in the ventricles, atria, or both. Our observations support this pathophysiologic construct, as we found that the magnitude of the rise in BNP levels after stress testing was related to the degree of inducible ischemia. Even after adjusting for differences in demographics, cardiac history, medications, exercise performance, and ejection fraction, the degree of myocardial ischemia was independently associated with the magnitude of increase in BNP levels. We also found that baseline BNP levels before stress testing were elevated in patients who subsequently devel-

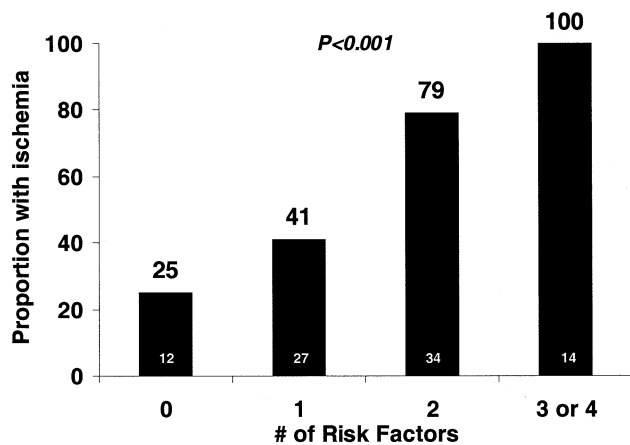


Figure 7. Proportion with inducible ischemia among patients categorized by the number of risk factors (male gender, limiting angina, ST-segment depression, post-stress test B-type natriuretic peptide level ≥ 80 pg/ml). Numbers within each bar represent the number of patients in that group. The p value is for trend across categories.

oped myocardial ischemia. This relationship was similar when restricted to patients with a normal ejection fraction and no clinical history of heart failure. This observation may reflect the effects of chronic or repetitive ischemia on BNP synthesis and release.

Our findings are supported by data from several other studies. The Heart and Soul Study found that patients with baseline BNP levels ≥ 105 pg/ml (a cut point similar to the baseline levels seen in our patients with severe inducible ischemia) were at a two-fold increased risk of having a new wall motion abnormality on exercise echocardiography (15). In terms of acute changes in circulating BNP levels, in a study of 70 individuals who were categorized on the basis of angiography, those with documented CAD had higher levels of BNP during exercise than those without (12). In a recent study of patients with angiographically confirmed CAD, the average increase in BNP with exercise across the entire study population was 18 pg/ml (16). It has also been shown that BNP levels rise after coronary angioplasty, but not coronary angiography, an observation ascribed to myocardial ischemia due to transient coronary occlusion with balloon inflation (17).

Although larger than many of the previous studies, our study is still relatively small. However, it was an unselected population that reflected typical patients presenting for stress testing. We relied on nuclear imaging to quantify the degree of ischemia. This remains an imperfect gold standard. Nonetheless, we think it superior to angiography alone, which can verify the presence of coronary atherosclerosis but cannot demonstrate inducible ischemia. The magnitude in rise of BNP was relatively small, and the interquartile ranges were wide. However, by design, the duration of significant ischemia during stress testing is brief. Moreover, other factors, including heart failure, can contribute to an elevated BNP. Our goal was not to demonstrate the utility of a "stress BNP," but rather to study the release

patterns of cardiac natriuretic peptides in response to transient myocardial ischemia. The ability of BNP levels to add to the diagnostic utility of stress testing needs to be confirmed in additional studies.

In summary, we found that transient myocardial ischemia was associated with an immediate increase in circulating BNP levels and, to a lesser extent, NT-pro-BNP levels, and that the degree of rise was proportional to the severity of ischemia. These findings confirm an important pathophysiologic link between the severity of an acute ischemic insult and the level of circulating BNP, and thus support the incorporation of BNP into multimarker strategies for risk stratification to gain insight into ventricular dysfunction associated with myocardial ischemia (18).

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REFERENCES

- Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med* 1998;339:321-8.
- Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 2002;347:161-7.
- Omland T, Aakvaag A, Bonarjee VV, et al. Plasma brain natriuretic peptide as an indicator of left ventricular systolic function and long-term survival after acute myocardial infarction: comparison with plasma atrial natriuretic peptide and N-terminal proatrial natriuretic peptide. *Circulation* 1996;93:1963-9.
- Morita E, Yasue H, Yoshimura M, et al. Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction. *Circulation* 1993;88:82-91.
- de Lemos JA, Morrow DA, Bentley JH, et al. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med* 2001;345:1014-21.
- Morrow DA, de Lemos JA, Sabatine MS, et al. Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-segment elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI-18. *J Am Coll Cardiol* 2003;41:1264-72.
- Sundsfjord JA, Thibault G, Larochelle P, Cantin M. Identification and plasma concentrations of the N-terminal fragment of proatrial natriuretic factor in man. *J Clin Endocrinol Metab* 1988;66:605-10.
- Jonckheere AR. A distribution-free k-sample test against ordered alternatives. *Biometrika* 1954;41:133-45.
- Hino J, Tateyama H, Minamino N, Kangawa K, Matsuo H. Isolation and identification of human brain natriuretic peptides in cardiac atrium. *Biochem Biophys Res Commun* 1990;167:693-700.
- Wijbenga JA, Balk AH, Boomsma F, Man in't Veld AJ, Hall C. Cardiac peptides differ in their response to exercise: implications for patients with heart failure in clinical practice. *Eur Heart J* 1999;20:1424-8.
- Nishimura K, Ban T, Saito Y, Nakao K, Imura H. Atrial pacing stimulates secretion of atrial natriuretic polypeptide without elevation of atrial pressure in awake dogs with experimental complete atrioventricular block. *Circ Res* 1990;66:115-22.

12. Marumoto K, Hamada M, Hiwada K. Increased secretion of atrial and brain natriuretic peptides during acute myocardial ischaemia induced by dynamic exercise in patients with angina pectoris. *Clin Sci (Lond)* 1995;88:551-6.
13. Mizuno Y, Yoshimura M, Harada E, et al. Plasma levels of A- and B-type natriuretic peptides in patients with hypertrophic cardiomyopathy or idiopathic dilated cardiomyopathy. *Am J Cardiol* 2000;86:1036-40.
14. Ikeda T, Matsuda K, Itoh H, et al. Plasma levels of brain and atrial natriuretic peptides elevate in proportion to left ventricular end-systolic wall stress in patients with aortic stenosis. *Am Heart J* 1997;133:307-14.
15. Bibbins-Domingo K, Ansari M, Schiller NB, Massie B, Whooley MA. B-type natriuretic peptide and ischemia in patients with stable coronary disease: data from the Heart and Soul study. *Circulation* 2003;108:2987-92.
16. Marie PY, Mertes PM, Hassan-Sebbag N, et al. Exercise release of cardiac natriuretic peptides is markedly enhanced when patients with coronary artery disease are treated medically by beta-blockers. *J Am Coll Cardiol* 2004;43:353-9.
17. Tateishi J, Masutani M, Ohyanagi M, Iwasaki T. Transient increase in plasma brain (B-type) natriuretic peptide after percutaneous transluminal coronary angioplasty. *Clin Cardiol* 2000;23:776-80.
18. Sabatine MS, Morrow DA, de Lemos JA, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation* 2002;105:1760-3.