

Exercise – induced changes of B-type natriuretic peptide uncover the unknown coronary artery disease in patients with chest pain and normal left ventricular systolic function.

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Background-Aim: The diagnostic and prognostic utility of B-type natriuretic peptide (BNP) has been demonstrated in patients either with heart failure or with known coronary artery disease (CAD). We aimed to investigate the utility of the exercise-induced changes of BNP in patients with chest pain, unknown CAD and normal left ventricular systolic function.

Methods and Results: We evaluated 100 consecutive patients (mean age 58.7 ± 9) years (80% male) with left ventricular ejection fraction $>50\%$. Blood samples were collected and BNP was measured before exercise stress testing, at peak and 20 min following it. All patients underwent coronary artery angiography.

Univariate analysis, showed that a 1.3 fold increment of BNP values, (from before to peak exercise BNP values), have 11 times greater odds of having CAD. ROC analysis revealed a sensitivity of 81.8% and a specificity of 71.8%. Interestingly, patients showing 1.79 times increment of before to peak exercise BNP values have 19 times greater odds of having multi-vessel disease with a sensitivity of 81% and specificity of 81.8% . Multi-variate analysis revealed that BNP increment from before to peak exercise remained statistically significant regardless of the presence of other risk factors for atherosclerosis.

Conclusion: The exercise-induced changes of BNP in patients with chest pain, normal left ventricular systolic function and unknown CAD can uncover patients with CAD and discriminate those with angiographically severe one.

Key words: Coronary artery disease, B-type natriuretic peptide, exercise

Reduced myocardial blood flow results in a cascade beginning with relaxation failure, rise in filling pressure, progressing to contraction abnormalities, ECG changes and finally symptoms.(1) Myocardial ischemia impairs both systolic and diastolic left ventricular function leading to an increase of myocardial wall stress that is the primary stimulus for B-natriuretic peptide secretion (2,3). B-type natriuretic peptide seems to be helpful in the diagnosis, prognosis and therapy not only in the setting of heart failure patients but also in patients with known coronary artery disease (4-13). Accordingly, it has been suggested that in patients with stable coronary artery disease resting-BNP values are associated with the presence of myocardial ischemia and angiographic disease severity (4-6). Moreover, it has been consistently shown that resting-BNPs are powerful predictors of death in patients with stable coronary artery disease or acute coronary syndrome (12-14). Thus, the association between the resting-BNP values and ischemia has been thoroughly demonstrated (15). However, patients with stable coronary artery disease (or even with unknown coronary artery disease) may also experience ischemia in their daily life (16) pointing out the role of exercise – induced changes of BNPs. In this respect, it has been suggested that in patients with known coronary artery disease (17), the exercise – induced changes of BNP is an important link to myocardial ischemia. (18) This has been confirmed by other investigators although coronary angiography is missing (19).

Therefore, we aimed to investigate whether, in patients with unknown coronary artery disease presented with chest pain and normal left ventricular systolic function, the exercise – induced changes of B-type natriuretic peptide can uncover, angiographically proved, coronary artery disease.

Methods

Study patients. We evaluated 105 consecutive patients with chest pain referred to outpatient clinic of our hospital. Exclusion criteria were impaired left ventricular ejection fraction $< 50\%$, history of coronary artery disease, left ventricular hypertrophy, the presence of more than mild valvular heart disease, rhythm disturbances, and more than mild renal insufficiency (serum creatinine > 2 mg/dl). One hundred consequently patients with no evidence of acute coronary artery syndrome who met the above mentioned criteria underwent treadmill exercise stress testing. Blood samples were collected before, at peak and 20 min following exercise stress testing. All patients underwent coronary artery angiography 48 to 72 hours after exercise. The results of each examination were analyzed by two independent experts on the respective field, unaware of clinical or other data. The protocol was approved by the ethical committee of our hospital and written informed consent was obtained from all patients.

Exercise ECG

All patients underwent a multistage treadmill exercise test with the standard Bruce protocol. Criteria for test interruption were: diagnostic ST-segment shift ≥ 2 mm, extreme fatigue or dyspnea, systolic blood pressure ≥ 240 mm Hg, diastolic blood pressure ≥ 120 mm Hg, or maximal age-predicted heart rate. The exercise ECG was considered to be diagnostic for ischemia if there was ≥ 1 mm flat or downsloping ST-segment depression 0.08 seconds after the J point.

Serial BNP measurements

Blood samples were collected and BNP was measured before (time-point 1), at peak (time-point 2) and 20 min after exercise (time-point 3). (20) Hence their difference (Δ) and their ratio as well, were calculated.

All samples were collected in plastic EDTA tubes and frozen at -70°C until the time of analysis. BNP testing was performed using the Triage BNP assay (Biosite Diagnostic, San Diego California). The lowest detectable measurement for this assay is 5pg/ml whereas the highest is 3000pg/ml. Inter-assay coefficients of variation were 8% to 15% ; intra-assay coefficients were 6% to 8%.

Coronary angiography.

Coronary angiography was performed according to standard techniques by the Judkins approach. A significant coronary artery stenosis was defined as $\geq 70\%$ luminal stenosis in one or more major epicardial coronary arteries.

Statistical Analysis

Categorical data were summarized as frequencies or percentages. Continuous data were summarized as mean \pm Standard Deviation (S.D). We used t-test for independent samples to compare means of continuous variables and chi-square test for qualitative variables. We used the Kolmogorov-Smirnov test for normality in order to evaluate assumption of t-test. Logarithmic transformation of non-normally distributed variables was used. Mann-Whitney test was used to compare BNP changes (Delta values). Repeated-measures ANOVA was used to detect statistically significant changes in BNP levels between groups. Receiver operating curve (ROC) analysis using AUC (Area under curve) was performed for discrimination between groups. $P < 0.05$ was considered statistically significant. Data analysis was performed with SPSS software, version 13.0.

Results

Coronary angiography revealed significant coronary artery stenosis in 78 patients (78%), who formed the CAD group, whereas the remaining patients formed the non-CAD group. In the CAD group, 41 patients (52%) had one-vessel disease, 22 (28%)

two-vessel disease, and 15 (20%) three-vessel disease, including three patients with significant left main stenosis. Demographic characteristic of the whole study group as well as of non-CAD and CAD groups are shown in Table 1. An ECG positive treadmill exercise test was observed in 41 of 72 patients (57%) in the CAD group compared with 5 of 28 patients (18%) in the non-CAD group ($p<0.001$). Thus, exercise stress testing had a 57% sensitivity and 52% specificity. In the whole study group mean BNP values before exercise were 20.4 ± 16.0 pg/dl, which at peak increased to 62.9 ± 61.9 pg/dl and to 96.7 ± 110.9 pg/dl 20min after exercise ($p<0.001$).

CAD vs non-CAD group

BNP values in both CAD and non-CAD group were measured at three different time-points; before exercise (1), at peak (2), and 20 min after it (3). BNP values at every single time-point differed between CAD and non-CAD group (Table 2, Figure 1), at a statistical significance level ($p=0.001$) being progressively apart over time (p for interaction=0.046).

BNP values at different time-point

The difference (Δ) of BNP values at different time-point (Δ 2-1, Δ 3-1, Δ 3-2) are shown in Table 2. The exercise – induced increase of BNP value was greater in the CAD group compared to the non-CAD group ($p=0.01$). Accordingly, the Δ 3-1 was greater in the CAD group compared to the non-CAD group ($p=0.028$), indicating a larger amount of BNP release during exercise in the CAD group.

ROC analysis (Figure 2) for discrimination between CAD and non-CAD group showed a borderline significance for the change of BNP values, from before to after exercise (time-point 3 / time-point 1 ratio), with an optimal cut-off value of 2.73 fold increment (area under the curve 0.636, 95% CI 0.500-0.772, $p=0.05$) with sensitivity 59.7% and specificity 77.37%. The change of BNP values from peak to after exercise

(time-point 3 / time-point 2 ratio), did not manage to differentiate the groups. Concerning the change of before to peak exercise (time-point 2 / time-point 1 ratio), the ROC analysis showed an optimal cut-off value of an 1.3 fold increment of before to peak exercise BNP values (area under curve 0.672, 95% CI 0.532-0.813, $p=0.014$). This cut-off value had a sensitivity of 81.8% and a specificity of 71.8%. Univariate logistic regression analysis revealed that patients having more than an 1.3 fold increment of before to peak exercise BNP values had 11 times greater odds of having CAD disease (OR= 11.45 with 95% CI 3.48-37.66, $P<0.001$). This cut-off value remained a significant predictor of CAD disease after adjustment for age, gender, smoking status, presence of hypertension, diabetes mellitus, hypercholesterolemia and familiar history of CAD (OR=18.59 with 95% CI 4.14-83.45, $p<0.001$).

Coronary Artery Disease Severity

We further divided our study group according to the number of diseased vessels, characterizing as severe coronary artery disease (multi-vessel) those patients having lumen artery stenosis $>70\%$ in 2 or/and 3 epicardial vessels (angiographically proved). Thus the presented sub-groups are: non-CAD group ($n=22$ patients), one-vessel disease group ($n=41$ patients), multi-vessel disease ($n=37$ patients). Table 3 shows serial BNP measurements for each group.

Non CAD vs single-vessel disease

Even if each BNP value differed between non-CAD and single-vessel group at different time-points (overall $p=0.008$), serial BNP values changed at the same way (p for interaction= 0.486). Thus, there was no difference of exercise – induced increase of BNP (23.9 ± 47.7 pg/ml for non-CAD vs 36.07 ± 51.5 pg/ml for single-vessel group $p=0.248$). Regarding the Δ 3-1 no difference was found between the non-CAD group and one-vessel group (37.6 ± 59.9 pg/ml vs 61.0 ± 88.59 pg/ml, respectively, $p=0.365$).

Similarly, no difference was found in Δ 3-2 BNP values (13.6 ± 28.3 pg/ml vs 23.97 ± 55.94 pg/ml, respectively, $p=0.752$). The ROC curve did not manage to differentiate one-vessel and non-CAD group based on the change of BNP values from before to peak exercise (time-point 2 / time-point 1 ratio), [area under curve 0.617 (95% CI 0.462-0.772, $p=0.130$).

Multi-vessel disease v.s non-CAD

Each BNP value differed between groups at different time-point (overall $p < 0.001$), while serial BNP values change at a different manner (p for interaction = 0.012). Thus, there was a statistically significant difference of exercise – induced increase (Δ 2-1) of BNP (23.94 ± 47.77 pg/ml vs 61.41 ± 58.74 pg/ml, $p < 0.001$) between the non-CAD and multi-vessel group. Similarly, a statistically significant difference was found in Δ 3-1 between the non CAD group and multi-vessel group (37.60 ± 59.95 pg/ml vs 116.13 ± 130.78 pg/ml, respectively, $p=0.002$) and in Δ 3-2 BNP values (54.72 ± 106.91 pg/ml v.s 13.66 ± 28.35 pg/ml, respectively, $p=0.042$).

ROC analysis for discrimination between multi-vessel disease versus non CAD patients showed an optimal cut-off value of 1.79 times increment of before to peak exercise (point-time 2 / point-time1 ratio) BNP values [area under curve 0.751 (95% CI 0.600-0.901, $p=0.001$). This cut-off value had a sensitivity of 81.0 % and specificity of 81.8%. Univariate logistic regression analysis revealed that patients having more than 1.79 times increment of BNP between peak and baseline values had 19 times greater odds of having multi-vessel disease (OR= 19.28 with 95% CI 4.95-75.17, $p < 0.001$). This cut-off value remained a significant predictor of CAD disease after adjustment for age, gender, smoking status, presence of hypertension, diabetes mellitus and family history (OR=37.52 with 95% CI 3.91-359.33, $p=0.02$).

Discussion

The present study demonstrates that in patients with chest pain and normal left ventricular function, serial BNP measurements; before at peak and 20 min after exercise, are consistently higher in patients with coronary artery disease. Interestingly, the fold increment of before to peak exercise BNP values can uncover patients with coronary artery disease and discriminate those with angiographically severe one.

Serial BNP measurements

Throughout the exercise protocol (from before to 20 min after exercise), BNP values were consistently increased in both CAD and non-CAD groups, being more pronounced in the former group. This is in keeping with previous reports where it has been shown that the elevation of BNP that occurs during exercise is more pronounced in patients with ischemia. (21). Accordingly, it has been suggested that elevated levels of BNP at rest (22) as well as after stress (23) are associated with the presence of coronary artery disease and myocardial ischemia (19). This is the case even in the absence of left ventricular dysfunction (as in our study) since myocardial ischemia augment cardiac BNP and pro-BNP gene expression leading to increased plasma BNP and pro-BNP concentrations (24). Although some studies reported that BNP returned to baseline values 10-15 min after exercise (21) in the present study this was not observed (different sample population characteristics), showing however the dynamic nature of natriuretic peptide release and confirming that exercise induced changes of BNP are multi-factorial (including volume and pressure changes as well as changes in neutral endo-peptidase activity or BNP kinetics). This dynamic nature of BNP release has been previously demonstrated, at a median time 7 min after exercise, by showing an increase of BNP values in both ischemic and non-ischemic group (7).

Although there are several reports suggesting the importance of BNP measurement in patients with stable coronary artery disease, to the best of our knowledge, this is the first study investigating the role of serial BNP values in patients with unknown coronary artery disease presented with chest pain and normal left ventricular function. Indeed, the results of the present study suggest that regardless of the presence of other risk factors, patients showing a 1.3 fold increment (before to peak exercise) BNP values, have 11 times greater odds of having coronary artery disease presenting a sensitivity of 81.8% and a specificity of 71.8%. Similar results have been reported in patients with previous myocardial infarction (19) or provoked ischemia (7). Accordingly, other studies found a rise in BNP during transient ischemia and during acute coronary syndrome (25-12). BNP has also been tested during dobutamine-induced myocardial ischemia showing a high predictive value for ischemia (26).

BNP values and coronary artery disease severity

The widespread application of BNP measurement in the setting of heart failure is very well established, whereas there is a growing evidence that BNP measurement can be also applied in patients with coronary artery disease. The results of the present study shows that patients showing 1.79 times increment of BNP values (before to peak exercise) have 19 times greater odds of having multi-vessel disease with a sensitivity of 81% and specificity of 81.8%. Investigators have suggested diastolic dysfunction as the cause of elevated natriuretic peptides when this elevation could not be attributed to impaired LV systolic function (27) (all patients of the present study had normal left ventricular ejection fraction). Accordingly, the BNP behaviour comes together with coronary artery disease severity, indicating that pathological conditions associated with diastolic dysfunction undoubtedly do raise natriuretic peptides levels (28) since myocardial ischemia may produce diastolic dysfunction (29) Thus, it is

likely that some cases of diastolic dysfunction and specifically of elevated natriuretic peptides in the absence of LV systolic dysfunction may result from coronary ischemia. Indeed, in the first report linking BNP to diastolic dysfunction all subjects had ischemic heart disease (27).

Clinical Implications

The determination of the exercise – induced increment of BNP value seems to be important in order to exclude coronary artery disease in patients with chest pain and normal left ventricular function. Indeed, both BNP and NT pro-BNP have strong predictive value in patients with stable coronary artery disease and display in predicting all-cause mortality (30). Accordingly, it has been shown that in patients with stable coronary artery disease BNP and LV ejection fraction were the strongest predictors for future cardiovascular events independent of known risk factors (31).

Conclusion

The exercise – induced changes of B-type natriuretic peptide in patients with chest pain, normal left ventricular systolic function and unknown coronary artery disease can uncover patients with coronary artery disease and discriminate those with angiographically severe one.

Table 1. Demographic characteristics and Exercise Stress Testing

	Whole study Group n=100	CAD group (n=78)	Non-CAD (n=22)	P value
Age (years)	58.7±9	58.8 ±9.0	58.3 ±9.4	0.815
Gender male(%)	80 (80%)	64(82%)	16(73%)	0.334
Smoking	40 (40%)	30(38.5%)	10(45.5%)	0.554
Hypercholesterolemia	66 (66%)	66(84.6%)	15(68.2%)	0.083
Hypertension	66 (66%)	50(64.1%)	16(72.7%)	0.451
Diabetes Mellitus	56 (56%)	44 (56.4%)	12 (54.5%)	0.876
Family history for CAD	58 (58%)	49(62.8%)	9(40.9%)	0.066

Data are presented as mean ±SD or number of patients, percentages are given in parentheses, p value represent comparison between coronary artery disease (CAD) and non-CAD group.

Table 2. Serial B-type Natriuretic Peptide (BNP) measurements; before exercise (time-point 1), at peak (time-point 2) and 20min after it (time-point 3), in both coronary artery disease (CAD) group and non-CAD group. The difference between time-points is also presented.

Groups	BNP (pg/dl) Before Exercise	BNP (pg/dl) Peak Exercise	BNP (pg/dl) After Exercise	p value
CAD group	21.8±15.3	69.9±63.2	109.5±117.5	<0.001§
Non-CAD group	14.2±17.0	38.2±51.1	51.8±68.9	<0.001§
p value	0.007	0.001	0.002	
	Difference (Δ) Peak(2)-Before(1)	Difference (Δ) After(3)-before(1)	Difference (Δ) After(3)- Peak(2)	
CAD group	48.09±56.17	87.5±113.57	38.75±85.21	NA
Non-CAD group	23.95±47.78	37.61±59.95	13.66±28.35	NA
p value	0.01	0.028	0.202	NA

§Repeated measures ANOVA, NA= Non Applicable

Table 3. Serial BNP measurements ; before exercise, at peak and 20min after it, in patients with no coronary artery disease (CAD), with single, and multi-vessel disease.

Groups	BNP (pg/dl) Before Exercise	BNP (pg/dl) Peak Exercise	BNP (pg/dl) After Exercise	p-value
No CAD (n:22)	14.2±17.0	38.2±51.1	51.8±68.9	0.002
Single Vessel (n:41)	22.9±15.5	59.0±55.5	84.33±90.4	<0.001
Multi-vessel (n:37)	20.65±15.22	82.05±69.64	136.78±137.2	<0.001
p value	0.02	0.001	0.001	

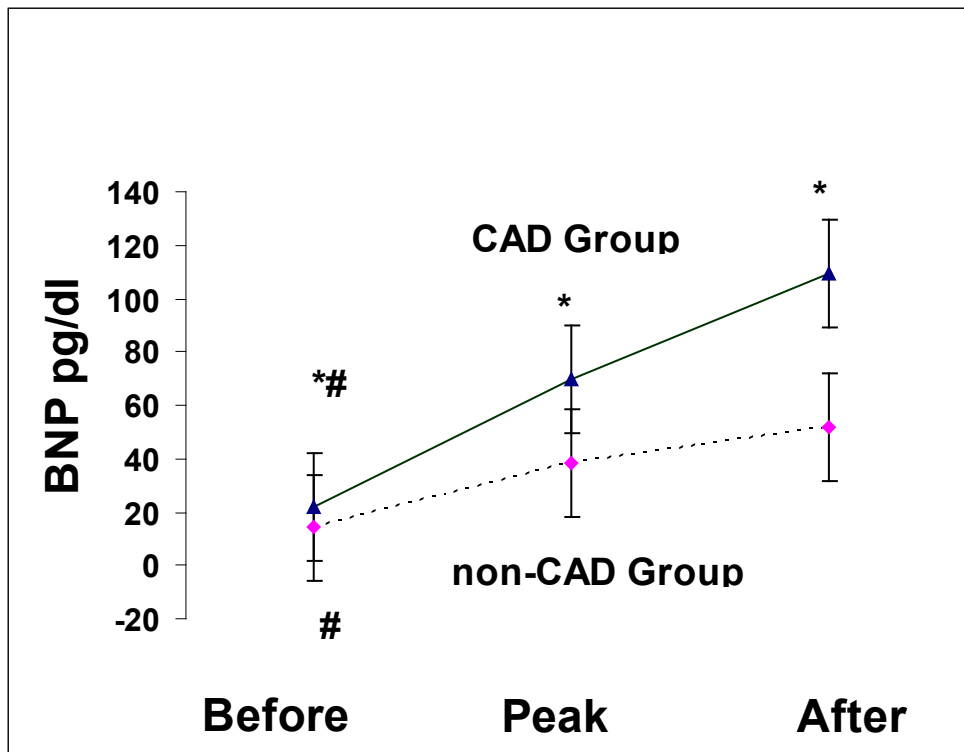


Figure 1. BNP values before, at peak and after exercise in the coronary artery disease (CAD) and non-CAD group. * and # represent p values <0.05 of BNP values between the groups and within the same group respectively. For more details see also text and Table 2.

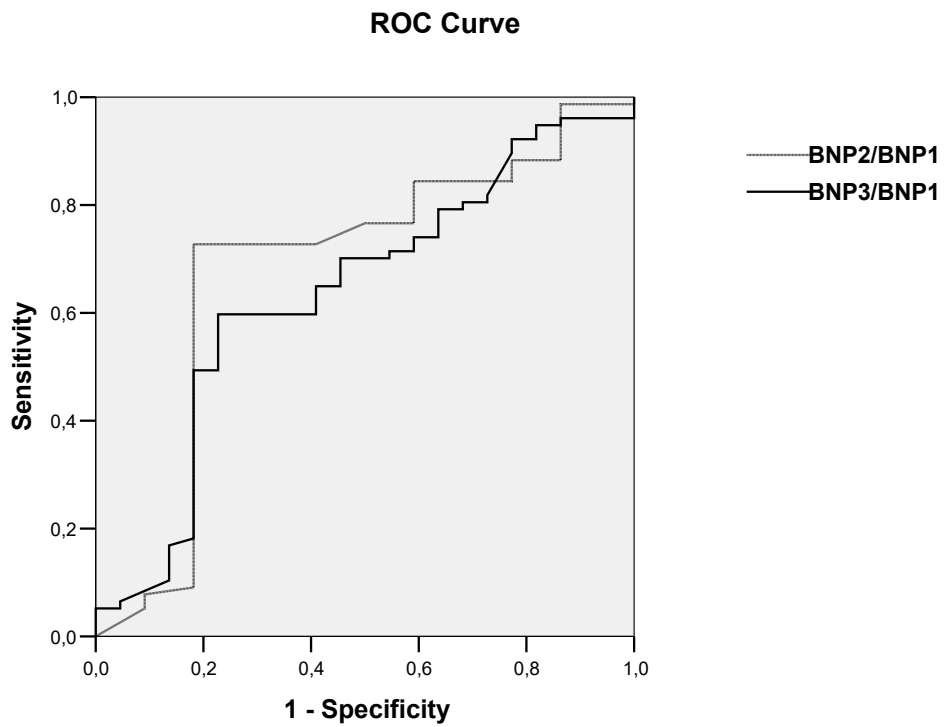


Figure 2. . BNP Receiver Operating Curve (point-time 2 / point-time1 ratio and point-time 3 / point-time 1 ratio) discriminate coronary artery disease patients.

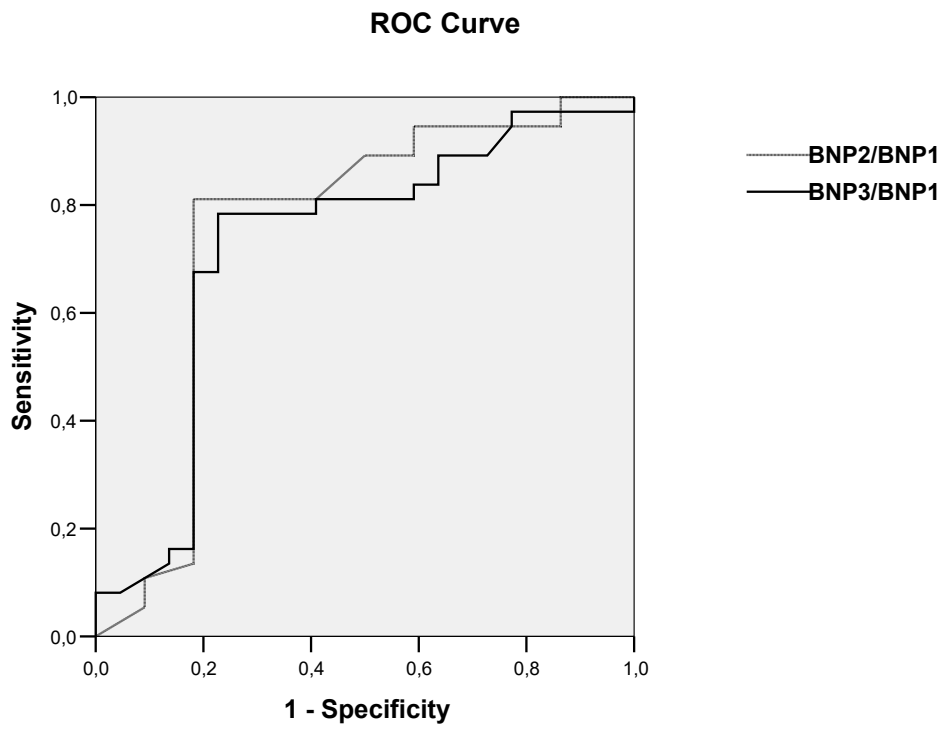


Figure 3. BNP Receiver Operating Curve (point-time 2 / point-time1 ratio and point-time 3 / point-time 1 ratio) discriminate multi-vessel coronary artery disease patients.

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