Baseline plasma N-terminal pro-B-type natriuretic peptide is associated with the extent of stress-induced myocardial ischemia during dobutamine stress echocardiography

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Objective **To determine the relationship between baseline plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and the presence and extent of myocardial ischemia during dobutamine stress echocardiography (DSE).**

Methods **NT-proBNP was measured in 170 consecutive patients prior to DSE. Rest wall motion abnormalities (RWMAs) and new wall motion abnormalities (NWMAs) were scored using a 5-point, 17-segment model. Kruskal–Wallis tests were applied to study differences in NT-proBNP levels between patients with normal DSE, RWMAs but no NWMAs, and NWMAs, and (in patients with NWMAs) between those with 1–2, 3–4 and >4 ischemic segments. Univariate and multivariate regression analyses were used to determine the value of NT-proBNP in predicting NWMAs.**

Results **The median NT-proBNP level was 110 ng/l (interquartile range: 42–389 ng/l). Median NT-proBNP was 59, 321 and 440 ng/l in patients with normal DSE, with RWMAs but no NWMAs, and with NWMAs, respectively ($P<0.0001$). Among patients with NWMAs, median NT-proBNP was associated with the number of ischemic segments: 364, 710 and 2376 ng/l in patients with 1–2, 3–4 and >4 ischemic segments, respectively ($P<0.001$). Elevated NT-proBNP levels were significantly associated with NWMAs (odds ratio per 100 ng/l increase: 1.14, 95% confidence interval: 1.1–1.2) in a multivariate analysis of clinical baseline variables and RWMAs.**

Conclusion **Elevated baseline levels of NT-proBNP are associated with the presence and extent of myocardial ischemia during DSE, independent of the presence of RWMAs.** ***Coron Artery Dis*** **17:255–259 © 2006 Lippincott Williams & Wilkins.**

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Introduction

Plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a neurohormone, synthesized in the ventricular myocardium and released in response to ventricular wall stress [1–3]. NT-proBNP is produced as a prohormone that is enzymatically cleaved into brain-type natriuretic peptide and NT-proBNP [4]. NT-proBNP is a strong marker for the diagnosis, severity and prognosis of patients with heart failure [5,6]. More recently, NT-proBNP was shown to have prognostic value in patients with acute coronary syndromes [7–11].

Limited information is available on the association between NT-proBNP levels and the presence and extent of stress-induced myocardial ischemia. Dobutamine stress echocardiography (DSE) is a widely used non-invasive technique for the diagnosis and prognosis of coronary artery disease [12]. Patients who experience transient new wall motion abnormalities (NWMAs) during DSE, a specific marker of myocardial ischemia, may also experience transient ischemic episodes during daily life.

We conducted a prospective study to assess the relationship between pre-test NT-proBNP levels and the presence and extent of stress-induced myocardial ischemia in patients undergoing DSE.

Methods

Study population

The study included consecutive patients with suspected or known coronary artery disease who were referred for DSE at the Thorax Center, Rotterdam, The Netherlands, between October 2003 and December 2004. Patients

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with severe valvular heart disease or hypertrophic or dilated cardiomyopathy were excluded. The hospital's Medical Ethical Committee approved the study protocol. On the basis of hospital records and personal interviews at the time of stress testing, a medical history, including clinical risk factors, medications, prior history of myocardial infarction, angina pectoris, congestive heart failure, coronary artery revascularization, stroke and transient ischemic attacks, was recorded and a baseline 12-lead electrocardiogram was obtained. We have chosen to include the six patients with renal failure into our multivariate analysis; we have adjusted for renal failure. Patients were characterized as having diabetes mellitus if they were treated with insulin or if they had a pre-test fasting plasma glucose level $\geq$ 126 mg/dl ($\geq$ 7.0 mmol/l). Hypercholesterolemia was recorded if patients presented with a plasma cholesterol level of $\geq$ 212 mg/dl ($\geq$ 5.5 mmol/l) or if patients were taking lipid-lowering agents. Hypertension was recorded if patients presented with a blood pressure of 140/90 mmHg or higher or if patients received antihypertensive medication. Smoking included only current smoking. Renal failure was recorded if patients presented with a serum creatinine level $\geq$ 2.0 mg/dl ($\geq$ 177 µmol/l) or in those who required dialysis.

Measurement of plasma N-terminal pro-B-type natriuretic peptide

Immediately before the performance of DSE, a heparinized venous blood sample was collected for measurement of NT-proBNP level. Venous blood samples were centrifuged and plasma was frozen at -80°C until assay. NT-proBNP was measured with an electrochemiluminescence immunoassay kit (Elecsys 2010; Roche GmbH, Mannheim, Germany). The method is a 'sandwich'-type quantitative immunoassay based on polyclonal antibodies against epitopes in the N-terminal part of proBNP. The lower detection limit was 5 ng/l. Intra-assay coefficients of variance at 271 and 6436 ng/l were 1.9 and 0.9%, respectively. A reference value of NT-proBNP was determined in 100 control patients prior to the start of the study. In these patients, the median (interquartile range) NT-proBNP level was 19 ng/l (12–4.37 ng/l) in male patients and 37 ng/l (27–52 ng/l) in female patients. Assays were performed by a laboratory technician blinded to the patient's clinical and stress test data [13].

Dobutamine stress echocardiography

Patients underwent a resting two-dimensional echocardiographic examination from the standard apical and parasternal views. Images were digitally stored and were also recorded on videotape. A 12-lead electrocardiogram was recorded. Dobutamine hydrochloride was then administered intravenously by infusion pump, starting at 5 µg/kg per min for 3 min, followed by 10 µg/kg per min for 3 min and increasing by 10 µg/kg per min every 3 min to a maximum of 40 µg/kg per min (stage 5). The dobutamine infusion was stopped if a target heart rate (85% of a theoretic maximal heart rate [men: (220 – age) × 85%; women: (200 – age) × 85%] was achieved. If the target heart rate was not achieved and patients had no symptoms or signs of ischemia, atropine sulfate (starting with 0.25 mg, increased to a cumulative maximum of 2.0 mg) was given intravenously at the end of stage 5 while dobutamine administration was continued. During the test, a 12-lead electrocardiogram was recorded every minute. Blood pressure was measured by sphygmomanometry every 3 min. Metoprolol was administered to reverse the side effects of the administration of dobutamine or the dobutamine–atropine combination if the side effects did not revert spontaneously and quickly. The criteria for stopping the test were (1) severe new echocardiographic wall motion abnormalities in multiple locations, (2) horizontal or down-sloping electrocardiographic ST depression of $\geq$ 0.2 mV measured 80 ms after the J point, or ST-segment elevation of $\geq$ 0.2 mV in the absence of Q waves, (3) symptomatic decline in systolic blood pressure of more than 40 mmHg from the resting value, or a systolic blood pressure of less than 100 mmHg, (4) hypertension (blood pressure $>$ 240/140 mmHg), (5) severe angina pectoris and (6) intolerable adverse effects considered to be the result of dobutamine or atropine.

Wall motion analysis

Offline assessment of echocardiographic images was made by two experienced investigators without knowledge of the patient's clinical data (D.P., J.J.B. and S.E.K.). The left ventricle was divided into 17 segments and wall motion was scored on a 5-point scale (a score of 1 indicating normal, 2 mild hypokinesis, 3 severe hypokinesis, 4 akinesis and 5 dyskinesis). For each patient, a wall motion score index (total score divided by the number of segments scored) was calculated at rest and at peak heart rate. NWMAs during the stress test were considered diagnostic for ischemia. When there was disagreement between the two assessors, a third investigator viewed the images without knowledge of the previous assessments, and a majority decision was reached.

Statistical analysis

Continuous data were expressed as mean ($\pm$ SD) or median ($\pm$ interquartile range) (in skewed distributions), and compared using the Student *t*-test or the Mann–Whitney *U*-test, when appropriate. Patients were divided into three groups according to DSE results: group 1 normal DSE, group 2 RWMAs but no NWMAs and group 3 NWMAs. Patients with NWMA were further divided into patients with 1–2 ischemic segments, 3–4 ischemic segments and $>$ 4 ischemic segments. NT-proBNP values in patients with a normal DSE, with RWMAs but no NWMAs, and with NWMAs were compared using the Kruskal–Wallis test. The Kruskal–Wallis test was also used to compare NT-proBNP values in patients with 1–2, 3–4 and $>$ 4 ischemic segments.

Receiver operating curve analysis was used to determine the optimal cutoff value of NT-proBNP to predict NWMAs. Univariable logistic regression analysis was used to evaluate the crude value of NT-proBNP in predicting stress-induced myocardial ischemia. NT-proBNP level and clinical risk factors identified as confounders (significant univariate predictors of stress-induced myocardial ischemia) were included in a final multivariable logistic model. Odds ratios are given with 95% confidence intervals. For all tests, a *P*-value < 0.05 (two sided) was considered significant. All analyses were performed using SPSS 11.0 statistical software (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics

Inclusion criteria were fulfilled in 170 patients. Baseline characteristics are presented in Table 1. The median NT-proBNP level for all patients was 110 ng/l (interquartile range: 42–389 ng/l). The following clinical parameters were associated with increased levels of NT-proBNP: age above 70 years, angina pectoris, previous myocardial infarction, congestive heart failure, hypertension, renal failure, ST segment changes and Q waves on baseline electrocardiogram (Table 2).

Dobutamine stress echocardiography results

Heart rate increased from 73 ± 14 beats per minute at rest to 133 ± 17 beats per minute at peak stress. The maximum dose of dobutamine infusion was 36.8 ± 7 μg/kg per min. Atropine was administered in 52% of patients to achieve target heart rate. No fatal complications occurred during or immediately after the stress test. DSE was normal in 97 patients (57%). RWMAs but no NWMAs were detected in 45 patients (26%). NWMAs occurred in 28 patients (16%). The mean number of ischemic segments in patients with stress-induced myocardial ischemia was 3.2 ± 1.2.

Table 1 Baseline characteristics of 170 patients

Characteristic	Number (%) or mean (±SD)
Demographics	
Age (years)	59 ± 13
Male sex	120 (71%)
Cardiovascular history	
Angina pectoris	45 (26%)
Previous myocardial infarction	69 (41%)
History of congestive heart failure	39 (23%)
Previous coronary artery revascularization	16 (9%)
History of cerebrovascular accident	15 (9%)
Clinical risk factors	
Diabetes mellitus	31 (18%)
Hypercholesterolemia	70 (41%)
Hypertension	46 (27%)
Smoking	32 (19%)
Renal failure	6 (4%)
Abnormal baseline electrocardiogram[a]	70 (41%)
Cardiac medication use	
Aspirin	56 (33%)
ACE inhibitor	63 (37%)
Beta-blocker	109 (64%)
Calcium channel blocker	28 (16%)
Diuretic	49 (29%)
Nitrate	16 (9%)
Statin	87 (51%)

[a]Electrocardiography with one or more of the following: Q-waves consistent with a previous myocardial infarction, ST segment changes, left bundle branch block and right bundle branch block. ACE, angiotensin-converting enzyme.

Table 2 Median plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels in relation to patient characteristics

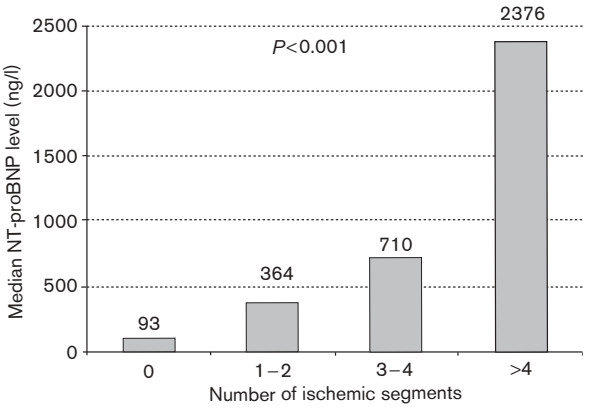
Characteristic	Median NT-proBNP level (interquartile range) +	Median NT-proBNP level (interquartile range) −	*P*-value
Age >70 years	12 (4–40)	25 (11–59)	0.04
Male sex	17 (5–51)	11 (4–29)	0.1
Angina pectoris	52 (23–84)	10 (4–26)	<0.001
Previous myocardial infarction	39 (13–81)	10 (4–22)	<0.001
History of congestive heart failure	40 (10–100)	11 (5–36)	0.007
History of cerebrovascular accident	33 (15–67)	12 (5–43)	0.08
Diabetes mellitus	38 (8–81)	12 (5–37)	0.2
Hypertension	29 (10–62)	11 (4–38)	0.03
Smoking	23 (4–49)	12 (5–46)	0.5
Hypercholesterolemia	13 (5–51)	13 (5–40)	0.9
Renal failure	172 (64–254)	12 (5–49)	0.04
Electrocardiography			
ST segment changes	53 (30–92)	12 (5–38)	0.004
Q-waves	37 (10–80)	11 (5–33)	<0.001
Left bundle branch block	51 (11–59)	12 (5–40)	0.2
Right bundle branch block	27 (7–46)	13 (5–46)	0.5

N-terminal pro-B-type natriuretic peptide levels and dobutamine stress echocardiography results

The median NT-proBNP level was 59 ng/l (interquartile range: 34–118) in patients with a normal DSE, 321 ng/l (interquartile range: 161–516) in patients with RWMAs but no NWMAs, and 440 ng/l (interquartile range: 195–1328) in patients with NWMAs ($P < 0.001$). In the group of patients with NWMA, NT-proBNP levels correlated with the extent (number of segments) of stress-induced myocardial ischemia (Fig. 1): the median level of NT-proBNP was 364 ng/l (interquartile range: 93–440), 710 ng/l (interquartile range 169–1565) and 2376 ng/l (interquartile range: 964–2554), in patients with 1–2 ($n = 10$), 3–4 ($n = 14$) and > 4 ($n = 4$) ischemic segments, respectively ($P < 0.001$).

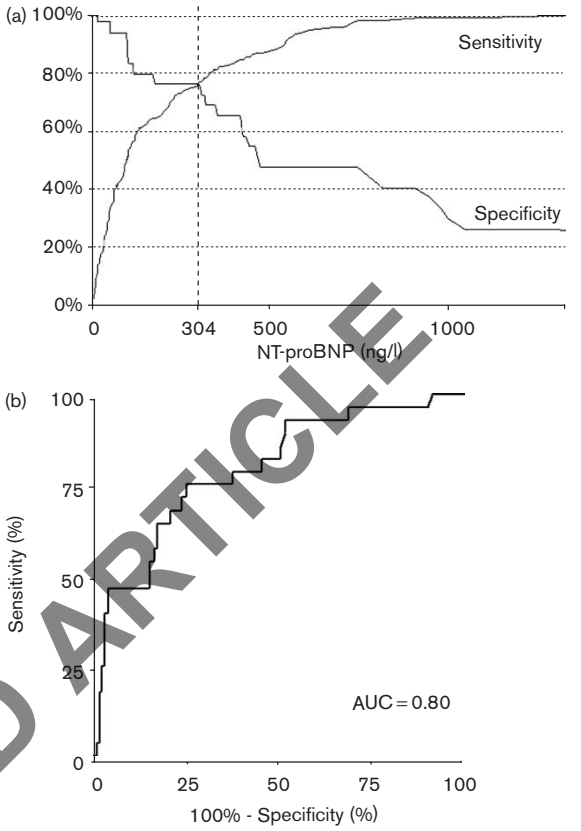
The optimal cutoff value of NT-proBNP to predict stress-induced myocardial ischemia was 304 ng/l, using receiver operating curve analysis (area under the curve 0.80) (Fig. 2). A cutoff value of 304 ng/l was associated with a sensitivity and specificity of 76%. Univariate associations of clinical parameters and NT-proBNP levels with stress-induced myocardial ischemia are presented in Table 3. After adjusting for RWMA and clinical risk factors, elevated NT-proBNP levels were independently associated with stress-induced myocardial ischemia (odds ratio for every 100 ng/l increase of NT-proBNP level: 1.14, 95% confidence interval: 1.10–1.18) (Table 4).

Fig. 1



Median plasma N-terminal pro-B-type natriuretic peptide levels (NT-proBNP) in relation to the number of ischemic segments during dobutamine stress echocardiography.

Fig. 2



Receiver operating curve analysis demonstrates that the optimal cutoff value of plasma N-terminal pro-B-type natriuretic peptide levels (NT-proBNP) for predicting new wall motion abnormalities was 304 ng/l (dashed line crossing at intersection of sensitivity and specificity) (a). The area under the curve (AUC) was 0.80 (b). The *x*-axis (NT-proBNP values) presents individual values and does not represent a linear or logarithmic scale.

Discussion

The present study was conducted to evaluate the relationship between baseline levels of NT-proBNP and the extent of stress-induced myocardial ischemia during DSE. The level of NT-proBNP was related to the presence and extent of myocardial ischemia, manifested as NWMAs. An elevated baseline NT-proBNP level was independently associated with stress-induced myocardial ischemia after adjustment for clinical data and RWMA. To our knowledge, this is the first study to demonstrate an independent association between baseline NT-proBNP levels and dobutamine stress-induced myocardial ischemia.

The human brain natriuretic peptide (BNP) gene, located on chromosome 1, encodes the prohormone proBNP, which is split into the 32 amino acid BNPs and the 76 amino acid NT-proBNPs [4]. NT-proBNP is synthesized predominantly in the left ventricular myocardium and released in response to ventricular wall stress, that is, ventricular dilatation and pressure overload [3,4]. The synthesis and release of natriuretic peptides in patients with coronary artery disease may be triggered by the effects of ventricular wall stress secondary to chronic or repetitive ischemia or by ischemia itself. It has been demonstrated in an experimental rat model of acute myocardial infarction that ventricular BNP mRNA expression and tissue concentrations of BNP were increased in both the non-infarcted and the infarcted regions [14]. Another published study, using myocardial biopsies from patients with coronary artery disease, has demonstrated an association between BNP mRNA expression in ischemic myocardium and plasma BNP levels, even in the absence of left ventricular dysfunction as evaluated by ventriculography [15].

Ndrepepa *et al.* [16] studied patients with angina pectoris and acute myocardial infarction, and found a positive association between the level of NT-proBNP and the severity of angiographic coronary artery disease. Sabatine *et al.* [17] measured NT-proBNP levels before and after exercise testing with nuclear perfusion imaging, and showed that NT-proBNP levels rose immediately in patients with exercise-induced transient myocardial ischemia and that the magnitude of NT-proBNP rise was associated with the severity of ischemia. It was unclear from their study whether baseline levels were independently associated with myocardial ischemia.

DSE is an established method for diagnosis of coronary artery disease [12,18,19]. Elevated NT-proBNP levels before stress testing suggest that patients with inducible ischemia during DSE often sustain spontaneous ischemic

Table 3 Univariate predictors of stress-induced myocardial ischemia

	OR	95% CI	*P*-value
NT-proBNP level per 100 ng/l increase	1.16	1.1–1.2	<0.001
Age >70 years	2.0	0.8–4.9	0.1
Male sex	2.9	0.9–8.9	0.06
Angina pectoris	2.6	1.1–5.9	0.03
History of myocardial infarction	2.7	1.2–6.2	0.02
History of congestive heart failure	2.6	0.9–7.8	0.07
History of cerebrovascular accident	1.8	0.5–6.0	0.4
Hypertension	1.3	0.6–3.2	0.5
Smoking	1.2	0.5–3.2	0.7
Hypercholesterolemia	0.6	0.3–1.5	0.3
Diabetes mellitus	1.3	0.5–3.6	0.6
Renal failure	11.6	2.0–66.8	0.006
Abnormal electrocardiogram[a]	3.7	1.5–8.7	0.003

[a]Electrocardiography with one or more of the following: Q-waves consistent with a previous myocardial infarction, ST segment changes, left bundle branch block and right bundle branch block. OR, odds ratio; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 4 Multivariate model to predict stress-induced myocardial ischemia

	OR	95% CI	*P*-value
NT-proBNP level per 100 ng/l increase	1.14	1.1–1.2	0.02
Age >70 years	2.2	0.8–6.3	0.1
Male sex	2.0	0.5–7.5	0.3
Angina pectoris	1.1	0.3–3.3	0.9
History of myocardial infarction	0.8	0.2–2.8	0.7
History of congestive heart failure	1.1	0.3–4.5	0.9
Renal failure	1.0	0.1–12.0	1.0
Abnormal baseline electrocardiogram[a]	1.5	0.4–5.3	0.5
Rest wall motion abnormalities	2.4	0.8–7.4	0.1

[a]Electrocardiography with one or more of the following: Q-waves consistent with a previous myocardial infarction, ST segment changes, left bundle branch block and right bundle branch block. OR, odds ratio; CI, confidence interval; NT-proBNP, plasma N-terminal pro-B-type natriuretic peptide.

episodes prior to stress testing. A low level of NT-proBNP was associated with a low incidence of ischemia. On the contrary, a high level was associated with a high incidence of ischemia. NT-proBNP levels may potentially be useful in determining the probability of an abnormal test and perhaps exempting patients with low or high probability of myocardial ischemia from stress testing. Considering the longer half-life time of NT-proBNP compared with BNP, NT-proBNP may be a superior screening marker in patients scheduled for stress testing. Nevertheless, more studies are needed to confirm this conclusion before recommending this approach in clinical practice.

In conclusion, patients with stress-induced myocardial ischemia during DSE had higher baseline levels of NT-proBNP than patients without ischemia. NT-proBNP level was a significant predictor of stress-induced myocardial ischemia, independent of clinical risk factors and RWMA. In addition, the extent of stress-induced myocardial ischemia was positively correlated to the level of NT-proBNP. Levels of NT-proBNP could stratify patients with regard to the probability of having inducible ischemia.

References

1 Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, *et al.* Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 1994; **90**:195–203.

2 Yoshimura M, Yasue H, Okumura K, Ogawa H, Jougasaki M, Mukoyama M, *et al.* Different secretion patterns of atrial natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. *Circulation* 1993; **87**:464–469.

3 Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med* 1998; **339**:321–328.

4 Hall C. Essential biochemistry and physiology of (NT-pro)BNP. *Eur J Heart Fail* 2004; **6**:257–260.

5 Gardner RS, Ozalp F, Murday AJ, Robb SD, McDonagh TA. N-terminal pro-brain natriuretic peptide. A new gold standard in predicting mortality in patients with advanced heart failure. *Eur Heart J* 2003; **24**:1735-1743.

6 Bettencourt P, Azevedo A, Pimenta J, Frioes F, Ferreira S, Ferreira A. N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients. *Circulation* 2004; **110**:2168–2174.

7 Richards AM, Doughty R, Nicholls MG, MacMahon S, Sharpe N, Murphy J, *et al.*, Australia–New Zealand Heart Failure Group. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: prognostic utility and prediction of benefit from carvedilol in chronic ischemic left ventricular dysfunction. Australia–New Zealand Heart Failure Group. *J Am Coll Cardiol* 2001; **37**:1781–1787.

8 Omland T, Aakvaag A, Bonarjee VV, Caidahl K, Lie RT, Nilsen DW, *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 pro-atrial natriuretic peptide. *Circulation* 1996; **93**:1963–1969.

9 de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, *et al.* The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med* 2001; **345**:1014–1021.

10 Schnabel R, Rupprecht HJ, Lackner KJ, Lubos E, Bickel C, Meyer J, *et al.* Analysis of N-terminal-pro-brain natriuretic peptide and C-reactive protein for risk stratification in stable and unstable coronary artery disease: Results from the AtheroGene Study. *Eur Heart J* 2005; **26**:241–249.

11 Kragelund C, Gronning B, Kober L, Hildebrandt P, Steffensen R. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. *N Engl J Med* 2005; **352**:666–675.

12 Poldermans D, Fioretti PM, Boersma E, Bax JJ, Thomson IR, Roelandt JR, *et al.* Long-term prognostic value of dobutamine-atropine stress echocardiography in 1737 patients with known or suspected coronary artery disease: a single-center experience. *Circulation* 1999; **99**:757–762.

13 Yeo KT, Wu AH, Apple FS, Kroll MH, Christenson RH, Lewandrowski KB, *et al.* Multicenter evaluation of the Roche NT-proBNP assay and comparison to the Biosite Triage BNP assay. *Clin Chim Acta* 2003; **338**:107–115.

14 Hama N, Itoh H, Shirakami G, Nakagawa O, Suga S, Ogawa Y, *et al.* Rapid ventricular induction of brain natriuretic peptide gene expression in experimental acute myocardial infarction. *Circulation* 1995; **92**:1558–1564.

15 Goetze JP, Christoffersen C, Perko M, Arendrup H, Rehfeld JF, Kastrup J, *et al.* Increased cardiac BNP expression associated with myocardial ischemia. *FASEB J* 2003; **17**:1105–1107.

16 Ndrepepa G, Braun S, Mehilli J, von Beckerath N, Vogt W, Schomig A, *et al.* Plasma levels of N-terminal pro-brain natriuretic peptide in patients with coronary artery disease and relation to clinical presentation, angiographic severity, and left ventricular ejection fraction. *Am J Cardiol* 2005; **95**:553–557.

17 Sabatine MS, Morrow DA, de Lemos JA, Omland T, Desai MY, Tanasijevic M, *et al.* Acute changes in circulating natriuretic peptide levels in relation to myocardial ischemia. *J Am Coll Cardiol* 2004; **44**:1988–1995.

18 Sawada SG, Segar DS, Ryan T, Brown SE, Dohan AM, Williams R, *et al.* Echocardiographic detection of coronary artery disease during dobutamine infusion. *Circulation* 1991; **83**:1605–1614.

19 Picano E. Stress echocardiography. *Expert Rev Cardiovasc Ther* 2004; **2**:77–88.