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Natriuretic Peptides as Markers of Mild Forms of Left Ventricular Dysfunction: Effects of Assays on Diagnostic Performance of Markers

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Background: **We compared the performance of different natriuretic peptides to diagnose mild forms of left ventricular dysfunction (LVD) and investigated the influence of measuring B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) with different assays on the diagnostic performance of these markers.**
Methods: **We measured BNP (Triage® BNP), NT-proBNP (Biomedica), and N-terminal pro-A-type natriuretic peptide (NT-proANP; Biomedica) in 130 consecutive patients (age range, 28–83 years) with clinically suspected mild LVD. In patients with sufficient sample volume, we measured BNP and NT-proBNP with additional assays (Shionoria and Roche, respectively).**
Results: **For identifying patients with mild systolic LVD, BNP and NT-proBNP were the best markers, with mean (95% confidence interval) areas under the curves (AUC) of 0.78 (0.63–0.89) and 0.75 (0.58–0.87), respectively. However, the diagnostic performance of NT-proANP [AUC, 0.64 (0.48–0.77)] was significantly worse than that of BNP ($P = 0.014$). Both BNP assays (Triage and Shionoria) and both NT-proBNP assays (Biomedica and Roche) performed equally well for the diagnosis of systolic LVD despite the poor agreement between NT-proBNP assays. In patients with isolated diastolic LVD, the diagnostic performance of the Triage BNP [AUC, 0.70 (0.56–0.81)] was significantly better ($P = 0.006$) than that of Biomedica NT-proBNP [0.49 (0.34–0.65)]. Furthermore, the performance of the Biomedica NT-proBNP assay was significantly worse ($P = 0.03$) than that of the Roche NT-proBNP assay for diagnosis of isolated diastolic LVD.**
Conclusions: **The performance of BNP for the diagnosis of systolic or diastolic LVD is not affected by the assay used, whereas the performance of NT-proBNP for the diagnosis of isolated diastolic LVD is assay dependent.**

Heart failure (HF)[3] is an important clinical problem with significant morbidity, mortality, and socioeconomic impact. The natural history of HF is as bad as those of many cancers, and the 5-year mortality for mild HF is as high as ~50% *(1)*. The prevalence of the disease in the elderly is high *(2)*. Most patients with HF are diagnosed as New York Heart Association (NYHA) class I and II (asymptomatic or mildly symptomatic patients) *(3)*. This is clinically relevant because the majority of these patients are currently underdiagnosed. However, it has been shown that treatment of these patients with angiotensin-converting enzyme inhibitors or beta-blockers substantially delays disease progression *(4, 5)*. Therefore, screening for HF in high-risk populations would be of clear benefit.

Among all investigated neurohormones and natriuretic peptides, B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) *(6–12)* are the best markers to rule out left ventricular dysfunction (LVD). Some studies have also proposed NT-pro-A-type natriuretic peptide (NT-proANP) as a useful marker for the diagnosis of LVD

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[3] Nonstandard abbreviations: HF, heart failure; NYHA, New York Heart Association; BNP, B-type natriuretic peptide; NT-proBNP; N-terminal pro-B-type natriuretic peptide; LVD, left ventricular function; NT-proANP, N-terminal pro-A-type natriuretic peptide; LVEF, left ventricular ejection fraction; CI, confidence interval; and AUC, area(s) under the curve(s).

(13–16). Yamamoto et al. *(9)* demonstrated that BNP is a more powerful marker of either left ventricular systolic dysfunction, left ventricular diastolic dysfunction, or left ventricular hypertrophy than is ANP or NT-proANP. A consistent finding of all reports is the excellent negative predictive value of BNP. Furthermore, BNP has a good negative likelihood ratio for diagnosis of LVD compared with standard clinical indices, such as clinical history, electrocardiogram, and chest x-ray *(17)*. These clinical results led to the development of numerous commercially available assays to determine different natriuretic peptide hormones *(18)*. However, different epitopes and fragments of the same analyte are detected by different assays, and cross-reactivities of antibodies with prohormone fragments may vary. Because natriuretic peptide assays are not standardized at present, clinical study results must be interpreted with caution when different assays are used.

The aims of this study were (*a*) to investigate which of the natriuretic peptides, BNP, NT-proBNP, or NT-proANP, performs best in the diagnosis of mild forms of LVD and (*b*) to investigate the impact of using different assays on the diagnostic performance of these natriuretic peptides.

Materials and Methods

PATIENTS

We investigated 130 consecutive patients (median age, 63.5 years; age range, 28–83 years) with clinically suspected mild LVD, which could be caused by either isolated diastolic or systolic LVD. Patients were classified according to the NYHA classification *(19)* and according to the recommendations of the task force of the American College of Cardiology and the American Heart Association (four stages) *(20)*. All patients were referred for routine coronary angiography between December 2000 and January 2001 to rule out substantial coronary artery disease. Patients gave written informed consent for blood sampling for natriuretic peptide measurements, and this study is consistent with the Declaration of Helsinki. All patients underwent left heart catheterization with left ventriculography. Additionally, a complete echocardiographic examination assessed all clinically relevant routine indices such as left ventricular ejection fraction (LVEF), regional systolic left ventricular function, diastolic function, left ventricular mass, and systolic pulmonary artery pressure. These examinations were performed by experienced cardiologists who were blinded to the natriuretic peptide results. Isolated diastolic LVD was defined according to the guidelines of the European Society of Cardiology *(21)* as an age-adjusted pathologic mitral valve diastolic inflow pattern on Doppler echocardiography together with an increased left ventricular end-diastolic pressure ≥16 mmHg in the presence of a normal LVEF (>50% in two-dimensional echocardiography). This precluded misclassification based on higher age alone. All patients with diastolic dysfunction showed the pattern of impaired relaxation in echocardiography. Systolic dysfunction was graded by use of the echocardiographically determined LVEF. Patients were grouped into three classes based on the following criteria: mild systolic LVD was defined as a LVEF of 40–50% on two-dimensional echocardiography, moderate LVD was defined as a LVEF of 30–40% on echocardiography; and severe LVD was defined as a LVEF <30%. Forty-seven patients with neither systolic nor diastolic LVD served as age- and sex-matched controls.

For the clinical study on the diagnostic performance of markers in suspected mild LVD, 44 patients were excluded for the following reasons: 6 patients had moderate to severe LVD; 31 patients had a myocardial infarction within 2 weeks of blood withdrawal; 5 patients presented with renal diseases; and 2 patients underwent a high-dose corticosteroid pretreatment for contrast-agent allergy. The final study population for this clinical investigation comprised 86 individuals (Table 1). All patients of this population had calculated systolic right ventricular pressures (by echocardiography) within the reference interval (<35 mmHg) and no evidence of right ventricular dysfunction on echocardiography. However, samples from all 130 patients were used for testing assay agreement of the different natriuretic peptides.

Blood was drawn into EDTA-containing plastic tubes after a standardized period of rest (10 min) in a supine position. After blood withdrawal, samples were stored at 4 °C (up to 1 h) until measurement of BNP in whole blood (Triage® BNP); subsequently samples were centrifuged at 2000*g* for 10 min at 4 °C, and the plasma was stored below −20 °C for up to 1 month for later determination of BNP, NT-proBNP, and NT-proANP by the different assays. The study design was prospective with respect to measurement of BNP, NT-proBNP, and NT-proANP for the clinical evaluation of the diagnostic performances of these different natriuretic peptides and retrospective with respect to the measurement of these peptides with different assays for assay comparison. Because of limited sample volumes, not every sample could be tested with all assays (see the *Results*). All patient samples were analyzed with the Triage BNP, Biomedica NT-proBNP, and Biomedica NT-proANP assays; subsequently, if the sample volume was sufficient, samples were analyzed with the Roche NT-proBNP and finally with the Shionoria BNP assay.

ASSAYS

BNP was measured with the Triage BNP Test (Biosite Diagnostics) as described previously *(22)*. This assay uses a murine Omniclonal® antibody bound to the fluorescent label and a murine monoclonal antibody against the mono-disulfide bond-mediated ring structure of BNP-32. This monoclonal antibody is bound to the solid phase (personal communication by the manufacturer).

In addition, BNP was measured by a commercially available IRMA (cat. no. IC-1049; Shionoria), which does not need plasma extraction procedures as described pre-

Table 1. Patient characteristics.[a]

	Controls (group 1)	Isolated diastolic LVD (group 2)	Mild systolic LVD (group 3)	*P* values between groups
n	47	20	19	NS[b]
Male, n (%)	30 (64)	15 (75)	14 (74)	NS
Mean (SD) age years	60.2 (12.1)	65.6 (8.7)	67.7 (9.6)	NS
NYHA	0–2[c]	0–2	0–2	1 vs 3: $P < 0.001$
Asymptomatic (class 0), n (%)	20 (43)	6 (30)	1 (5)	
NYHA class 1, n (%)	26 (55)	10 (50)	11 (58)	
NYHA class 2, n (%)	1 (2)[c]	4 (20)	7 (37)	
Stages A–D	A	B and C	B and C	1 vs 2: $P < 0.001$
Stage A, n (%)	47 (100)			1 vs 3: $P < 0.001$
Stage B, n (%)		17 (85)	12 (63)	
Stage C, n (%)		3 (15)	7 (37)	
Mean (SD) diseased vessels	0.8 (0.9)	1.2 (1.2)	1.3 (1.2)	NS
No significant CAD, n (%)	23 (49)	8 (40)	6 (32)	
1-Vessel disease, n (%)	14 (30)	5 (25)	7 (37)	
2-Vessel disease, n (%)	8 (17)	3 (15)	1 (5)	
3-Vessel disease, n (%)	2 (4)	4 (20)	5 (26)	
History of AMI, n (%)	10 (21)	7 (35)	12 (63)	1 vs 3: $P = 0.001$
Diabetes mellitus type 2, n (%)	5 (11)	1 (5)	5 (26)	NS
Hypertension, n (%)	26 (55)	15 (75)	13 (68)	NS
Mean (SD) creatinine, μmol/L	93.2 (17.0)	90.3 (15.8)	102.5 (22.5)	NS
Drugs, n (%)				
ASA	44 (94)	19 (95)	16 (84)	NS
Beta-blockers	21 (45)	9 (45)	11 (58)	NS
ACE inhibitors	14 (30)	11 (55)	15 (79)	1 vs 3: $P < 0.001$
AT II receptor antagonists	0	2 (10)	2 (11)	1 vs 2: $P = 0.028$; 1 vs 3: $P = 0.024$
Diuretics	7 (15)	4 (20)	7 (37)	1 vs 3: $P = 0.048$
Calcium antagonists	6 (13)	5 (25)	1 (5)	NS
Statins	22 (47)	10 (50)	11 (58)	NS
Echocardiographic and hemodynamic data, mean (SD)				
EF (%; left ventriculography)	68 (5)	69 (6)	51 (6)	1 vs 3: $P < 0.001$ 2 vs 3: $P = 0.015$
Myocardial mass, g/m^2 BSA	118 (30)	141 (44)	146 (43)	1 vs 3: $P = 0.015$
LVEDD, mm	49 (7)	51 (7)	54 (8)	1 vs 3: $P = 0.006$
LAD, mm	38 (5)	41 (5)	43 (7)	1 vs 3: $P = 0.004$
RVEDD, mm	25 (3)	24 (4)	27 (3)	1 vs 3: $P = 0.008$ 2 vs 3: $P = 0.006$
+dP/dt, mmHg/s	2100 (464)	2387 (511)	1841 (646)	1 vs 2: $P = 0.023$ 2 vs 3: $P = 0.008$

[a] Values in parentheses indicate percentage of total number of cases.

[b] NS, not significant; CAD, coronary artery disease; AMI, acute myocardial infarction; ASA, acetosalicylic acid; ACE, angiotensin-converting enzyme; AT II, angiotension II; BSA, body surface area; LVEDD, left ventricular end-diastolic diameter; LAD, left atrial diameter; RVEDD, right ventricular end-diastolic diameter.

[c] In these patients, HF symptoms were mimicked by noncardiac diseases.

viously *(23)*. The assay uses an antibody specific to the C-terminal structure (amino acids 27–32) immobilized on a bead and a ^{125}I-labeled antibody specific to the intramolecular ring structure of human BNP-32 (amino acids 14–21), respectively *(24)*.

NT-proBNP(8–29) was assayed by a competitive enzyme immunoassay (cat. no. BI-20852; Biomedica) that uses an antibody specific against NT-proBNP(8–29) as described previously *(25)*.

Additionally, NT-proBNP(1–76) was measured by a sandwich electrochemiluminescence immunoassay (Elecsys 1010; Roche Diagnostics) that uses polyclonal antibodies specific against the epitopes NT-proBNP(1–21) and NT-proBNP(39–50) as described previously *(26, 27)*.

NT-proANP(1–98) was measured by a sandwich enzyme immunoassay (cat. no BI-20892; Biomedica), which uses antibodies specific for distinct epitopes of proANP(1–98), as described previously *(28)*.

CORONARY ANGIOGRAPHY AND LEFT VENTRICULOGRAPHY

Coronary angiography and left ventriculography were performed via femoral artery access using the Seldinger puncture technique. A 6-French pigtail catheter was used

to measure left ventricular end-diastolic and end-systolic filling pressures, and the first derivative of left ventricular pressure (dP/dt). The ventriculogram was analyzed by use of a computer software package. Calculation of the end-diastolic and end-systolic left ventricular volumes and ejection fraction was performed by use of the right anterior oblique projection by the area-length method. The frame nearest to the R-wave peak in the electrocardiogram was used as the end-diastolic frame, and the frame with the smallest ventricular volume was taken to calculate the end-systolic volume. Left ventricular volumes were normalized to body surface area.

Coronary angiography was performed according to the Judgkin technique. The degree of stenosis of coronary vessels was assessed visually in several projections by an experienced invasive cardiologist. Stenosis >70% of the vessel diameter was classified as hemodynamically significant.

ECHOCARDIOGRAPHY

Each patient underwent a complete standardized echocardiographic examination using an Acuson ultrasound imaging system (Acuson Sequoia C256; Siemens) equipped with a 3.5-MHz transducer suitable for second harmonic imaging. Parasternal long- and short-axis views as well as four, two, and three long-axis chamber views were obtained. Left ventricular volumes and ejection fraction were measured from the two-dimensional apical four-chamber view by the area-length method (modified Simpson method). The end-diastolic thicknesses of the intraventricular septum and the left ventricular posterior wall were measured in a parasternal short-axis view using the M-mode technique, and the Penn formula was used to calculate left ventricular mass. Left ventricular mass and volumes were normalized to body surface area. Left ventricular diastolic filling was evaluated by pulsed-wave Doppler measurement of velocities of early and late ventricular diastolic filling (E- and A-wave), as well as the deceleration time of the E-wave. Right ventricular systolic pressure was estimated by measurement of the systolic retrograde blood flow velocity into the right atrium by the continuous wave Doppler technique.

STATISTICS

ROC plot analysis *(29)* was carried out to illustrate and compare the diagnostic performance of the different natriuretic peptides and assays. Spearman rank correlation coefficients were calculated. The Mann–Whitney *U*-test was used for group comparisons. Data are given as the mean (SD), or as median and interquartile range (25th and 75th percentiles) if more appropriate, and natriuretic peptide concentrations are given in ng/L. Assays were compared by use of Bland–Altman plots *(30)* with Analyze-it of the software package Microsoft Excel (Ver. 1.63). A P value <0.05 was considered to indicate statistical significance.

Results

NATRIURETIC PEPTIDE CONCENTRATIONS

BNP, NT-proBNP, and NT-proANP increased significantly with the clinical severity of HF symptoms (Table 2). BNP as measured by the Triage BNP assay [median, 146 ng/L (interquartile range, 47–209 ng/L)] and NT-proBNP as measured by the Biomedica NT-proBNP assay [3602 (2023–4517) ng/L] were significantly increased in patients with mild systolic LVD compared with controls (BNP, $P = 0.001$; NT-proBNP, $P = 0.002$) and patients with isolated diastolic LVD (Triage BNP, $P = 0.026$; Biomedica NT-proBNP, $P = 0.011$; Fig. 1, A and B). Additionally, in patients with isolated diastolic LVD Triage BNP concentrations [37 (22–81) ng/L; $P = 0.018$; Fig. 1A] showed significant increases compared with controls. By contrast, NT-proANP concentrations were not significantly increased in either patients with mild LVD or patients with isolated diastolic LVD (Fig. 1C).

COMPARISON OF MARKERS

Mild systolic LVD. In patients with mild systolic LVD, the Triage BNP and Biomedica NT-proBNP showed comparable diagnostic performances (Fig. 2) with mean [95%

Table 2. Natriuretic peptide concentrations according to the NYHA classification and to the A–D stages of HF according to the American College of Cardiologists/American Heart Association task force.

	NYHA			Stage		
	Asymptomatic	Class 1	Class 2	A	B	C
BNP (Triage), ng/L						
Median	22	45	111	22	61	111
Interquartile range	8–39	12–133[a]	40–196[a]	8–48	20–160[a]	45–225[a]
NT-proBNP (Biomedica), ng/L						
Median	1595	2023	3286	1839	1882	3546
Interquartile range	1059–1916	1460–2830[a]	2426–4610[a,b]	1370–2411	1193–2782	2753–4785[a,b]
NT-proANP (Biomedica), pmol/L						
Median	2510	3489	3724	3333	3363	3920
Interquartile range	2080–3696	2638–5518[a]	2276–11 753[a]	2115–3950	2542–6165	2865–6212

[a] $P \leq 0.03$ between NYHA class 1 or 2 and asymptomatic individuals or between stage B or C and stage A of HF.
[b] $P \leq 0.03$ between NYHA class 1 and 2 or between stage B and C of HF.

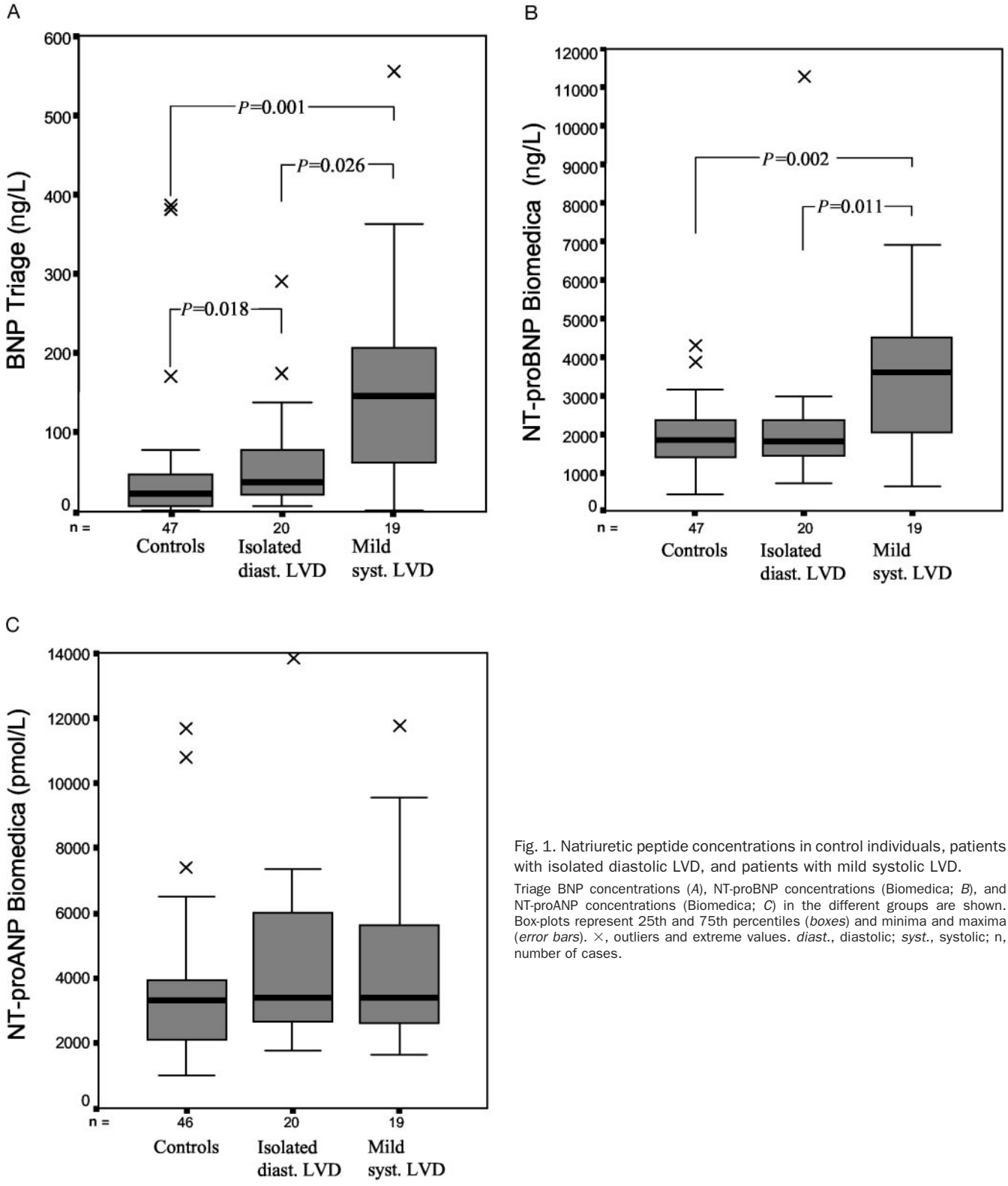

Fig. 1. Natriuretic peptide concentrations in control individuals, patients with isolated diastolic LVD, and patients with mild systolic LVD.

Triage BNP concentrations (*A*), NT-proBNP concentrations (Biomedica; *B*), and NT-proANP concentrations (Biomedica; *C*) in the different groups are shown. Box-plots represent 25th and 75th percentiles (*boxes*) and minima and maxima (*error bars*). ×, outliers and extreme values. *diast.*, diastolic; *syst.*, systolic; n, number of cases.

confidence interval (CI)] areas under the curves (AUC) of 0.78 (0.63–0.89) and 0.75 (0.58–0.87), respectively. However, NT-proANP gave a significantly ($P = 0.014$) smaller AUC [0.64 (0.48–0.77)] than Triage BNP; the AUC for NT-proANP was not significantly different from the AUC for NT-proBNP. The negative predictive values (95% CI)

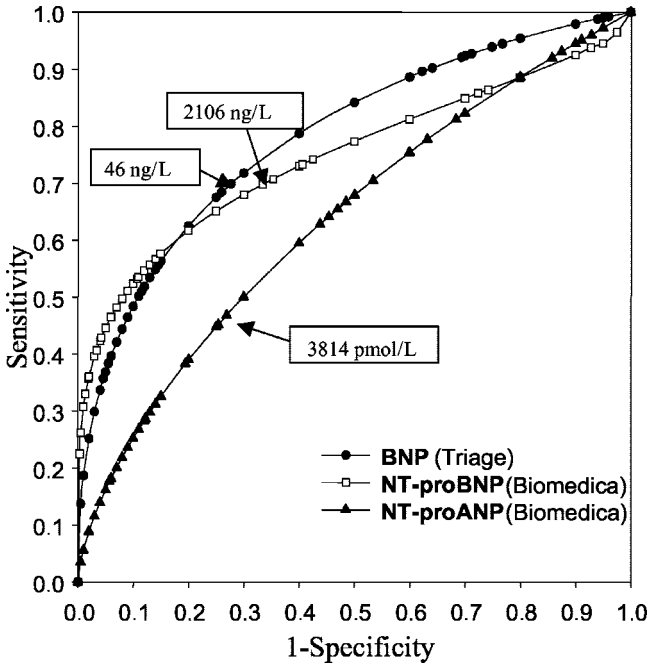

Fig. 2. ROC curves comparing the diagnostic performances of BNP (Triage; ●), NT-proBNP (Biomedica; □), and NT-proANP (Biomedica; ▲) for identifying patients with mild systolic LVD (n = 19 patients and 47 controls).

Optimum cutoff values are shown.

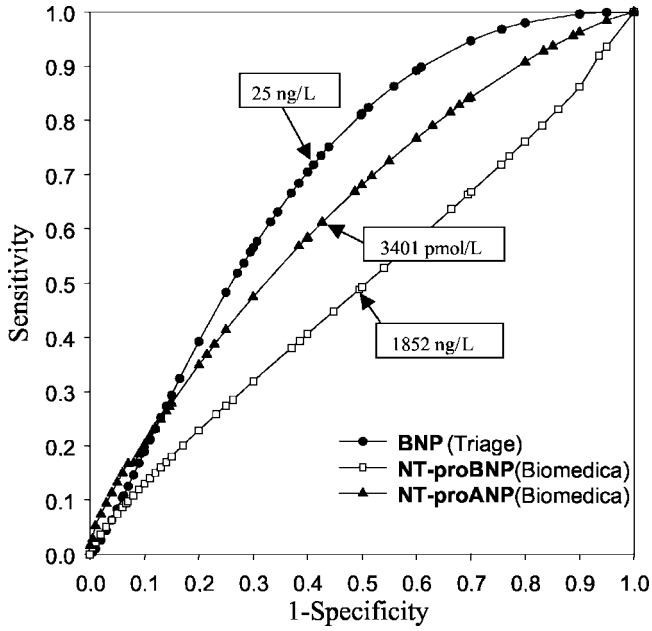

Fig. 3. ROC curves comparing the diagnostic performances of BNP (Triage; ●), NT-proBNP (Biomedica; □) and NT-proANP (Biomedica; ▲) for identifying patients with isolated diastolic LVD (n = 20 patients and 47 controls).

Optimum cutoff values are shown.

at optimal cutoff values (see Fig. 2) were 90 (76–97)% for BNP, 83 (67–94)% for NT-proBNP, and 77 (61–88)% for NT-proANP, respectively.

Isolated diastolic LVD. In patients with isolated diastolic LVD, the mean (95% CI) AUC for the Triage BNP (Fig. 3) of 0.70 (0.56–0.81) showed significantly better ($P = 0.006$) diagnostic performance than the AUC for the Biomedica NT-proBNP [0.49 (0.34–0.65)]. At the optimal cutoff value of 25 ng/L for Triage BNP, the sensitivity was 70 (46–88)%, the specificity was 57 (42–72)%, the positive predictive value was 41 (25–59)%, the negative predictive value was 82 (65–93)%, and the efficiency was 61 (49–73)%. NT-proANP had a smaller mean AUC of 0.63 (0.48–0.76), but was not significantly different from the Triage BNP.

correlations of natriuretic peptides with each other and with hemodynamic data

We found close correlations between the Triage BNP and Roche NT-proBNP ($r = 0.88$; $P < 0.001$), between the Shionoria BNP and Biomedica NT-proBNP ($r = 0.82$; $P < 0.001$), and between the Shionoria BNP and Roche NT-proBNP ($r = 0.88$; $P < 0.001$). The Triage BNP and Biomedica NT-proBNP correlated as well ($r = 0.78$; $P < 0.001$). Correlations between NT-proANP and the other natriuretic peptides were weak ($r = 0.34–0.51$; $P < 0.001$).

There were only weak correlations between natriuretic peptides and myocardial mass, atrial and ventricular dimensions, or hemodynamic data ($r = -0.052$ to 0.337; $P < 0.001–0.96$). The closest correlations were between the natriuretic peptides and LVEF obtained from left ventriculography (Triage BNP, $r = -0.459$; Biomedica NT-proBNP, $r = -0.376$; $P < 0.001$).

comparison of assays

BNP. In 81 individuals, the Triage BNP and Shionoria BNP assays showed a close correlation ($r = 0.96$; $P < 0.01$). Nevertheless, absolute BNP values measured with both assays differed markedly ($P < 0.001$), with concentrations measured by the Triage BNP being, on average, 110 ng/L higher (mean value of difference). However, there was better agreement of test results below concentrations of 100 ng/L (mean value of difference, 9.2 ng/L; Fig. 4A).

NT-proBNP. In 113 individuals, the NT-proBNP(8–29) assay (Biomedica) showed a moderate correlation with the NT-proBNP(1–76) assay (Roche; $r = 0.73$; $P < 0.01$). These assays also showed a marked concentration difference (see Fig. 4B) with a mean difference of 1803 ng/L ($P < 0.001$).

influence of measuring with different assays on the diagnostic performance of bnp and nt-proBNP

In a subgroup analysis, we compared the diagnostic performance of the Triage BNP and Shionoria BNP assays. There was equal diagnostic performance for both assays [mean (95% CI) AUC, 0.68 (0.49–0.84) for Triage BNP and 0.74 (0.56–0.88) for Shionoria; $P = 0.09$; Table 3]

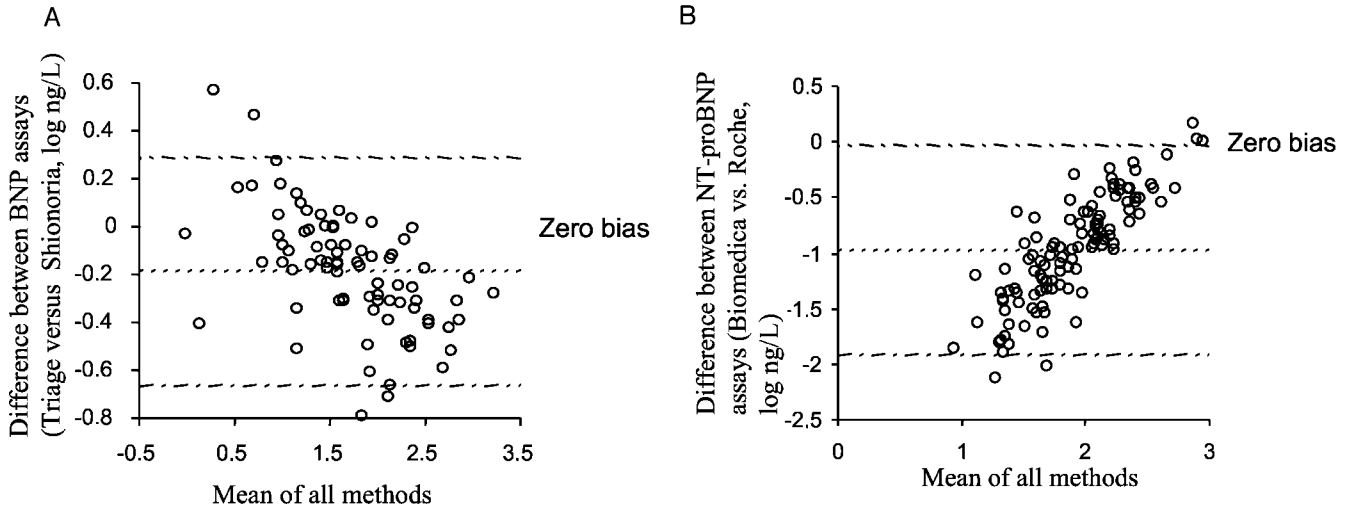

Fig. 4. Bland–Altman difference plots between the Triage and Shionoria BNP assays (*A*) and the Roche and Biomedica NT-proBNP assays (*B*).
Concentrations were log-transformed to exclude relationships between difference and magnitude and to achieve a gaussian distribution of values.

for the diagnosis of mild systolic LVD. In a further subgroup analysis, the diagnostic performance of the Biomedica and Roche NT-proBNP was compared. There was no statistically significant impact of assays on the ability of NT-proBNP to differentiate between controls and patients with mild systolic LVD (Table 4). However, in the comparison of the diagnostic performances of NT-proBNP in patients with isolated diastolic LVD, the diagnostic performance of the Biomedica NT-proBNP assay was significantly worse compared with the Roche NT-proBNP assay [$P = 0.03$; mean (95% CI) AUC, 0.44 (0.29–0.59) vs 0.58 (0.42–0.73); Table 4].

Discussion

In the present study, in contrast to several earlier published studies, we used a very exact definition of mild systolic LVD and isolated diastolic LVD based on several objective measurements *(19–21)*. Our controls were matched for age and sex and were very well characterized, showing normal echocardiographic, left ventriculographic, and left ventricular hemodynamic results. In agreement with our previous study *(8)*, BNP and NT-proBNP were interchangeable as diagnostic markers in patients with mild systolic LVD, whereas the diagnostic performance of NT-proANP was significantly worse than that of BNP. BNP and NT-proBNP concentrations were significantly increased in patients with mild systolic LVD compared with controls, independent of the assay used. In contrast, NT-proANP concentrations did not differ significantly from concentrations in controls. The lack of a significant increase in NT-proANP in mild systolic LVD is in contrast to some previous reports *(13, 15, 16)*. One explanation for this discrepancy is that there was less severe impairment of LVEF in our patients with systolic LVD. In the present study, the grading of the severity of LVD was not based solely on subjective individual symptoms, where LVEF can vary considerably in patients of a given NYHA class; objective data obtained from echocardiography and cardiac catheterization were also used to classify the severity of LVD. Furthermore, measurement of natriuretic peptide by less precise RIAs, which require extraction of plasma samples, may have influenced the results of earlier studies. However, our results agree very well with previous studies showing good performance of BNP and NT-proBNP compared with other natriuretic peptides or their second messenger, cGMP, in patients with impaired LVEF *(8, 9, 11, 12, 31, 32)*. In contrast to the results reported by Prontera et al. *(33)*, in our study the diagnostic performance of BNP and NT-proBNP was comparable. However, Prontera et al. could not exclude whether the differences in marker performance were just an effect of differences in assay precision. Our results showing the high negative predictive values of BNP and NT-proBNP confirmed results obtained in previous studies and indicate that these markers may be suitable tools to rule out mild systolic LVD in high-risk patients.

Using the European Society of Cardiology classifica-

Table 3. Comparison of the diagnostic performances of Triage and Shionoria BNP assays to identify patients with mild systolic LVD (n = 15 patients with mild systolic LVD and 27 controls).[a]

	BNP	
	Triage	Shionoria
Sensitivity, %	73 (45–92)	73 (45–92)
Specificity, %	85 (66–96)	74 (54–89)
PPV,[b] %	73 (45–92)	61 (36–83)
NPV, %	85 (66–96)	83 (63–95)
Efficiency, %	83 (65–91)	74 (58–86)
Mean AUC	0.68 (0.49–0.84)	0.74 (0.56–0.88)
Cutoff, ng/L	70	34

[a] Values in parentheses are the 95% CI.
[b] PPV, positive predictive value; NPV, negative predictive value.

Table 4. Comparison of the diagnostic performance of Biomedica and Roche NT-proBNP assays to identify patients with mild systolic LVD and with isolated diastolic LVD.[a]

	NT-proBNP			
	Mild systolic LVD		Isolated diastolic LVD	
	Biomedica	Roche	Biomedica	Roche
Sensitivity, %	60 (32–84)	73 (45–92)	63 (38–84)	63 (38–84)
Specificity, %	63 (47–78)	78 (62–89)	37 (22–53)	49 (33–65)
PPV,[b] %	38 (19–59)	55 (32–77)	32 (18–49)	36 (20–55)
NPV, %	81 (64–93)	89 (74–97)	68 (45–86)	74 (54–89)
Efficiency, %	62 (49–75)	77 (63–87)	45 (32–58)	53 (40–66)
Mean AUC	0.70 (0.49–0.85)	0.74 (0.56–0.87)	0.44 (0.29–0.59)	0.58 (0.42–0.73)
Cutoff, ng/L	2123	251	1531	96
No. of cases	56	56	60	60

[a] Values in parentheses are the 95% CI.
[b] PPV, positive predictive value; NPV, negative predictive value.

tion of isolated diastolic dysfunction based on echocardiographic and hemodynamic data, we confirmed the significant increase in BNP, as measured by the Triage BNP assay, reported previously by Lubien et al. *(34)*, who used only echocardiographic criteria. However, we did not observe a similarly high diagnostic performance of Triage BNP, which may be explained by the fact that our cohort did not include patients with restrictive filling patterns. The high negative predictive value of BNP (82%) in the present study confirms the results of a previous report *(35)* and underlines the accuracy of BNP as a rule-out marker even for isolated diastolic LVD. Thus, BNP is a promising marker for the diagnosis of isolated diastolic LVD as well. We found no significant difference in the diagnostic performance of BNP and NT-proANP for the diagnosis of isolated diastolic LVD, which confirms a previous report of increased ANP and BNP concentrations in diastolic LVD *(36)*.

We found significant correlations among all tested natriuretic peptides. In accordance with previous studies, we found only weak inverse correlations of LVEF and BNP *(7–9, 12, 37)*. There are only two published studies (in which LVEF was determined by magnetic resonance imaging) showing a close correlation between BNP and LVEF ($r = -0.78$) *(38)* and between NT-proBNP (Roche) and LVEF ($r = -0.75$) *(39)* in patients in NYHA classes II–IV. The more severely reduced LVEF than in our study population and the more precise method for the calculation of LVEF likely account for the closer correlations.

There was a close correlation between BNP measured by Biosite Triage and by Shionoria assay. However, Bland–Altman plots showed an acceptable agreement between methods only at concentrations <100 ng/L. There was no influence of the BNP assay used on the diagnostic performance of the marker. By contrast, the correlation between NT-proBNP measured by the Biomedica and Roche methods was only moderate, and Bland–Altman plots revealed only poor test agreement over the whole measuring range. Nevertheless, NT-proBNP assays were not significantly different in identifying patients with mild systolic LVD. However, in patients with isolated diastolic LVD, the AUC were significantly different. The epitopes detected by the different assay antibodies in the NT-proBNP molecule are different, which may influence diagnostic endpoints in very mild forms of LVD.

There are very limited data on the influence of measuring natriuretic peptides with different assays on diagnostic performance. In accordance with our results, Tjeerdsma et al. *(40)* and Fischer et al. *(22)* found a close correlation between Triage BNP and Shionoria BNP results, with Triage BNP values being higher than Shionoria values. However, the AUC for the assays were higher in both studies compared with our AUC for BNP, which can be explained in part by either the more severely diseased patient cohort or by a younger control group not age-matched to the LVD patient group. Our data confirm the lack of influence of the assay used on the diagnostic performance of BNP in less severe LVD. Data regarding the recently Food and Drug Administration-cleared Centaur (Bayer) BNP assay showed a high correlation with the Shionoria as well as with the Triage BNP assays in a large multisite study *(24)*. The close correlation with the Shionoria assay is not surprising because the antibodies are identical in both assays *(24)*. However, the slope of 0.78 between the Centaur (Bayer) and the Triage (Biosite) BNP assays showed that these assays did not agree very well. Nevertheless, similar to our results, there was a high agreement at a cutoff value of 100 ng/L. For the Roche and Biomedica methods, a recent report demonstrated a similar lack of assay and analytical agreement with a large mean concentration difference between the NT-proBNP assays similar to that seen in our study *(41)*.

Our results for differentiating patients with isolated diastolic LVD from controls showed better performance of the Roche assay compared with the Biomedica assay, similar to that seen in the previous study for differentiating patients with asymptomatic structural heart disease

from individuals without. Both assays did not differ significantly in their diagnostic performances for the diagnosis of symptomatic LVD. The commercially available Triage BNP and NT-proBNP (Roche) assays were compared with different locally developed in-house RIAs for BNP and NT-proBNP *(42)*. Close correlations between all assays were found, but data on analytical assay agreement were not published. We found higher diagnostic efficiency of BNP and NT-proBNP than did the authors of that study *(42)*, although we included only patients with mild forms of LVD who consequently had lower BNP and NT-proBNP concentrations.

In conclusion, our study confirms the usefulness of BNP and NT-proBNP and extends previous findings by directly comparing commercially available BNP and NT-proBNP assays in the same study population. Furthermore, our results highlight the diversity of the natriuretic peptide assays on the market. Thus it is difficult to compare study results that are based on different assays. Published decision limits are valid only for the particular assay used. Our study population was too small for additional subgroup analysis to calculate age- and sex-dependent decision limits, but from our results a cutoff of 50 ng/L (14 pmol/L) for the Triage BNP assay could be a good screening value to exclude LVD in high-risk patients.

The BNP Triage tests and NT-proBNP Elecsys assays were gifts from Biosite (Velizy, France) and Roche (Penzberg, Germany), respectively. BNP Shionoria assays were a gift from Bayer Diagnostics (Tarrytown, NY), and NT-proANP and NT-proBNP assays were partly provided free of charge from Biomedica (Vienna, Austria). The assay manufacturers had no influence on the study design, data analysis or interpretation, or the content of this report.

References

1. Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, Levy D. Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort. J Am Coll Cardiol 1999;33:1948–55.
2. Mosterd A, Hoes AW, de Bruyne MC, Deckers JW, Linker DT, Hofman A, et al. Prevalence of heart failure and left ventricular dysfunction in the general population—The Rotterdam Study. Eur Heart J 1999;20:447–55.
3. McDonagh T, Morrison CE, Lawrence A, Ford I, Tunstall-Pedoe H, McMurray JJV, et al. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. Lancet 1997; 350:829–33.
4. SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fraction. N Engl J Med 1992;327: 685–91.
5. Packer M, Cohn JN. Consensus recommendations for the management of chronic heart failure. Am J Cardiol 1999;83:1A–32A.
6. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin. New neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. Circulation 1998;97:1921–9.
7. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-proBNP): a new marker of cardiac impairment. Clin Endocrinol 1997;47:287–96.
8. Hammerer-Lercher A, Neubauer E, Muller S, Pachinger O, Puschendorf B, Mair J. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal pro-atrial natriuretic peptide in diagnosing left ventricular dysfunction. Clin Chim Acta 2001;310:193–7.
9. Yamamoto K, Burnett JC Jr, Jougasaki M, Nishimura RA, Bailey KR, Saito Y, et al. Superiority of brain natriuretic peptide as a hormonal marker of ventricular systolic and diastolic dysfunction and ventricular hypertrophy. Hypertension 1996;28:988–94.
10. Cowie MR, Struthers AD, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, et al. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet 1997;350:1349–53.
11. McDonagh TA, Robb SD, Murdoch DR, Morton JJ, Ford I, Morrison CE, et al. Biochemical detection of left-ventricular dysfunction. Lancet 1998;351:9–13.
12. Friedl W, Mair J, Thomas S, Pichler M, Puschendorf B. Natriuretic peptides and cyclic guanosine 3′,5′-monophosphate in asymptomatic and symptomatic left ventricular dysfunction. Heart 1996; 76:129–36.
13. Arnlov J, Lind L, Stridsberg M, Andren B, Lithell H. N-terminal atrial natriuretic peptide and left ventricular geometry and function in a population sample of elderly males. J Intern Med 2000;247:699–708.
14. Lerman A, Gibbons RJ, Rodeheffer RJ, Bailey KR, McKinley LJ, Heublein DM, et al. Circulating N-terminal atrial natriuretic peptide as a marker for symptomless left-ventricular dysfunction. Lancet 1993;341:1105–9.
15. Fruhwald FM, Fahrleitner A, Watzinger N, Fruhwald S, Dobnig H, Schumacher M, et al. Natriuretic peptides in patients with diastolic dysfunction due to idiopathic dilated cardiomyopathy. Eur Heart J 1999;20:1415–23.
16. Daggubati S, Parks JR, Overton RM, Cintron G, Schocken DD, Vesely DL. Adrenomedullin, endothelin, neuropeptide Y, atrial, brain, and C-natriuretic prohormone peptides compared as early heart failure indicators. Cardiovasc Res 1997;36:246–55.
17. Landray MJ, Lehman R, Arnold I. Measuring brain natriuretic peptide in suspected left ventricular systolic dysfunction in general practice: cross-sectional study. BMJ 2000;320:985–6.
18. Mair J, Hammerer-Lercher A, Puschendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure [Review]. Clin Chem Lab Med 2001;39:571–88.
19. Remme WJ, Swedberg K. Guidelines for the diagnosis and treatment of chronic heart failure. Task force for the diagnosis and treatment of chronic heart failure, European Society of Cardiology. Eur Heart J 2001;22:1527–60.
20. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al., American College of Cardiology/American Heart Association. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to revise the 1995 Guidelines for the Evaluation and Management of Heart Failure). J Am Coll Cardiol 2001;38:2101–13.
21. European Study Group on Diastolic Heart Failure. How to diagnose diastolic heart failure. Eur Heart J 1998;19:990–1003.

22. Fischer Y, Filzmaier K, Stiegler H, Graf J, Fuhs S, Franke A, et al. Evaluation of a new rapid bedside test for quantitative determination of B-type natriuretic peptide. Clin Chem 2001;47:591–4.

23. Ry SD, Clerico A, Giannessi D, Andreassi MG, Caprioli R, Iascone MR, et al. Measurement of brain natriuretic peptide in plasma samples and cardiac tissue extracts by means of an immunoradiometric assay method. Scand J Clin Lab Invest 2000;60:81–90.

24. Wu AHB, Packer M, Smith A, Bijou R, Fink D, Mair J, et al. Analytical and clinical evaluation of the Bayer ADVIA Centaur automated B-type natriuretic peptide assay in patients with heart failure: a multisite study. Clin Chem 2004;50:867–73.

25. Missbichler A, Hawa G, Woloszczuk W, Schmal N, Hartter E. Enzyme immunoassays for proBNP fragments (8–29) and (32–57). J Lab Med 1999;23:241–4.

26. Karl J, Borgya A, Gallusser A, Huber E, Krueger K, Rollinger W, et al. Development of a novel, N-terminal-proBNP (NT-proBNP) assay with a low detection limit. Scand J Clin Lab Invest 1999;59(Suppl 230):177–81.

27. Collinson PO, Barnes SC, Gaze DC, Galasko G, Lahiri A, Senior R. Analytical performance of the N terminal pro B type natriuretic peptide (NT-proBNP) assay on the Elecsys 1010 and 2010 analysers. Eur J Heart Fail 2004;6:365–8.

28. Missbichler A, Hawa G, Schmal N, Woloszczuk W. Sandwich ELISA for proANP 1–98 facilitates investigation of left ventricular dysfunction. Eur J Med Res 2001;6:105–11.

29. Zweig MH, Campell G. Receiver-operating characteristics (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–77.

30. Bland MJ, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.

31. Vasan RS, Benjamin EJ, Larson MG, Leip EP, Wang TJ, Wilson PW, et al. Plasma natriuretic peptides for community screening for left ventricular hypertrophy and systolic dysfunction. JAMA 2002;288:1252–9.

32. Omland T, Aakvaag A, Vik-Mo H. Plasma cardiac natriuretic peptide determination as a screening test for the detection of patients with mild left ventricular impairment. Heart 1996;76:232–7.

33. Prontera C, Emdin M, Zucchelli GC, Ripoli A, Passino C, Clerico A. Natriuretic peptides (NPs): automated electrochemiluminescent immunoassay for N-terminal pro-BNP compared with IRMAs for ANP and BNP in heart failure patients and healthy individuals. Clin Chem 2003;49:1552–4.

34. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction. Comparison with Doppler velocity recordings. Circulation 2002;105:595–601.

35. Krishnaswamy P, Lubien E, Clopton P, Koon J, Kazanegra R, Wanner E, et al. Utility of B-natriuretic peptide levels in identifying patients with left ventricular systolic or diastolic dysfunction. Am J Med 2001;111:274–9.

36. Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, et al. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. JAMA 2002;288:2144–50.

37. McGeoch G, Lainchbury J, Town GI, Toop L, Espiner E, Richards AM. Plasma brain natriuretic peptide after long-term treatment for heart failure in general practice. Eur J Heart Fail 2002;4:479–83.

38. Groenning BA, Nilsson JC, Sondergaard L, Kjaer A, Larsson HB, Hildebrandt PR. Evaluation of impaired left ventricular ejection fraction and increased dimensions by multiple neurohumoral plasma concentrations. Eur J Heart Fail 2001;3:699–708.

39. Groenning BA, Nilsson JC, Sondergaard L, Pedersen F, Trawinski J, Baumann M, et al. Detection of left ventricular enlargement and impaired systolic function with plasma N-terminal pro brain natriuretic peptide concentrations. Am Heart J 2002;143:923–9.

40. Tjeerdsma G, de Boer RA, Boomsma F, van den Berg MP, Pinto YM, van Veldhuisen DJ. Rapid bedside measurement of brain natriuretic peptide in patients with chronic heart failure. Int J Cardiol 2002;86:143–9.

41. Mueller T, Gegenhuber A, Poelz W, Haltmayer M. Comparison of the Biomedica NT-proBNP enzyme immunoassay and the Roche NT-proBNP chemiluminescence immunoassay: implications for the prediction of symptomatic and asymptomatic structural heart disease. Clin Chem 2003;49:976–9.

42. Lainchbury JG, Campbell E, Frampton CM, Yandle TG, Nicholls MG, Richards AM. Brain natriuretic peptide and N-terminal brain natriuretic peptide in the diagnosis of heart failure in patients with acute shortness of breath. J Am Coll Cardiol 2003;42:728–35.