Journal of Clinical Laboratory Analysis 20:227–232 (2006)

Comparison of BNP and NT-proBNP Assays in the Approach to the Emergency Diagnosis of Acute Dyspnea

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N-terminal pro-brain natriuretic peptide (NT-proBNP) and BNP measurement could have a significant role in differentiating dyspnea between cardiac or pulmonary origin in the emergency room. The development of new and different commercial assays for these B-type natriuretic peptides offers the possibility of improving and simplifying their measurements but this could be defaulted due to the differences in methodology and the lack of assay standardization. We compared four available methods of measuring NT-proBNP and BNP and evaluated their usefulness in diagnosing the causes of breathlessness in the emergency room. The correlation of BNP with different assays was strong with $r > 0.98$ ($P < 0.0001$). Comparison studies-between NT-proBNP and BNP procedures were in good agreement with $r > 0.87$. The area under the receiver-operating characteristic curve (ROC) for BNP or NT-proBNP for detecting any cardiac dysfunction was higher than 0.96 (95% CI). A BNP value of 116 pg/mL measurement with the Access BNP assay (Beckman Coulter Inc., Fullerton, CA), a BNP value of 79 pg/mL with Advia Centaur BNP assay (Bayer Diagnostics, Tarrytown, NY), and an NT-proBNP level of 817 pg/mL in Elecsys NT-proBNP assay (Roche Diagnostic, Mannheim, Germany), showed both high sensitivity (>92%) and high specificity (>93%). We have found that NT-proBNP and BNP present similar diagnostic accuracies for the differential diagnosis of dyspnea. J. Clin. Lab. Anal. 20:227–232, 2006.

Key words: brain natriuretic peptides; dyspnea; heart failure; respiratory disease method comparison

INTRODUCTION

B-type natriuretic peptides are cardiac neurohormones (1) secreted from ventricular myocytes in response to ventricular volume expansion and pressure overload, producing natriuresis and diuresis and relaxing vascular smooth muscle and inhibiting the renin-angiotensin-aldosterone system (2). Natriuretic peptides are synthesized as preprohormones that lose a signal peptide. The formed prohormones (proBNP) split enzymatically into an inactive NH_2-terminal fragment (NT-proBNP) and a biologically active COOH-terminal peptide (BNP) (3), which are secreted into the blood circulation in equimolecular amounts. However, NT-proBNP shows a higher concentration than BNP, because of its different clearance mechanisms (4). Both peptides can be measured by an automated or semi-automated immunoassay in the clinical laboratory.

Its potential clinical usefulness (5) is for screening heart disease (6), stratification of patients with congestive heart failure, detection of left ventricular systolic and diastolic dysfunction (7) as prognostic markers in cardiac disease (8), monitoring the therapy (9), and the differential diagnosis of dyspnea. Therefore, the European Society of Cardiology recommends testing these natriuretic peptides along with chest x-rays and electrocardiograms when evaluating patients with suspected heart failure (10).

In the emergency room, rapid and accurate differentiation of cardiac dysfunction from other causes of dyspnea leads to the immediate initiation of the appropriate therapy. Unfortunately, diagnosis is not easy because the symptoms and signs are neither sensitive nor specific and there is considerable overlapping with patients who present with pulmonary disease. Thus, B-type natriuretic peptides can be useful with the differential diagnosis. (11,12).

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Received 16 August 2005; Accepted 11 May 2006
DOI 10.1002/jcla.20146
Published online in Wiley InterScience (www.interscience.wiley.com).

There are different commercial assay methods (enzyme, chemiluminescence, fluorescence immunoassays, and immunoradiometric assays) for measuring these two peptides, although they have different technical characteristics and possess different decision limits.

The aim of this study was to evaluate the value of NT-proBNP and BNP in patients with acute dyspnea in the emergency room. In addition, we compared different commercially available assays for B-type natriuretic peptide measurements, and finally, we evaluated which of them showed the best correlation with clinical features.

MATERIALS AND METHODS

Natriuretics peptides were measured in plasma samples of patients (n = 75) who were admitted to the emergency room and had acute dyspnea as the primary symptom, and a control group (n = 25) formed by routine patients with no cardiac or respiratory causes who were included as reference. The total population included patients (67% men and 33% women) aged 75 ± 14.77 (mean $\pm$ SD) years old. This was a prospective study. Patients were diagnosed according to their symptoms and signs and the following clinical and laboratory procedures: physical examination, blood test, electrocardiography (ECG), chest x-radiography, and in some cases, echocardiography criteria (10). Clinicians involved in the diagnosis were blinded to the values of the natriuretic peptides obtained.

Samples were taken into ethylenediaminetetracetic (EDTA) or heparinized tubes, centrifuged for 10 min at 1,500 g, and the plasma was stored at –20°C before assaying NT-proBNP and BNP.

EDTA-plasma BNP was measured by the following immunoassays: Advia Centaur BNP assay (Bayer Diagnostics), Access BNP assay (Beckman), and Triage Meter (Biosite Corporation, San Diego, CA). The Advia Centaur BNP assay is a two-site dual-monoclonal immunochemiluminescent assay. Triage BNP test is a point-of-care analyzer that uses murine monoclonal bound to alkaline phosphatase and polyclonal antibodies in a immunochromatographic fluorescence assay. BNP measurement in the Access BNP assay uses the same reagents of the Triage test applied to the Access analyzer. In all these assays, an antibody is directed to the C-terminal part of the natriuretic peptide and the other is directed against the ring (biologically active) portion of BNP.

Heparin plasma NT-proBNP was measured by electrochemiluminescence with the Elecsys NT-proBNP assay (Roche), which uses two polyclonal antibodies that bind to the amino terminal part of NT-proBNP.

Brain natriuretic peptide assay results were compared and the relationships among procedures were studied by applying the Passing-Bablok nonparametric regression and the Pearson's correlation coefficient. A P value <0.05 was considered significant.

To compare the diagnostic accuracy of each test for NT-proBNP and BNP, receiver operating characteristic (ROC) analysis was performed. The area under the curves (AUC) for each parameter were compared and values for specificity and sensitivity were estimated.

RESULTS

Patients with dyspnea were categorized into two groups corresponding to patients with cardiac dysfunction and respiratory diseases, respectively (10). Heart failure and respiratory disease etiologies in the study population are shown in Table 1.

The distribution of BNP and NT-proBNP concentrations in the patient population studied is shown in Fig. 1. Patients with cardiac dysfunction had significantly higher BNP/NT-proBNP values than non-cardiac dyspnea patients and normal groups.

The comparison study of the results obtained with the Access BNP, Advia Centaur BNP, and Elecsys NT-proBNP assays is shown in Fig. 2. The correlation coefficients obtained were between 0.87 and 0.98. The regression analysis with a 0.95 confidence interval gives the statistical data showed in Table 2. Triage BNP assay was only assayed in 25 patients and showed similar results to those obtained with BNP assays ($r > 0.97$).

Results of the regression parameters reveal the existence of proportional and constant systematic errors; the results obtained by these methods are well correlated but not transferable.

The ability of BNP/NT-proBNP to detect abnormal cardiac function in patients with dyspnea was assessed with ROC analysis. The area under the ROC curve was 0.979 for NT-proBNP in Elecsys and 0.975/0.965 for BNP in Access/Centaur. There were no significant differences (<0.001) among the AUC's (Fig. 3).

TABLE 1. Causes of cardiac and respiratory disease in the study population

Cardiac patients (n = 45)	
Left ventricular systolic	12%
Ischemic	12%
Ventricular dilation	9%
Valvar	15%
Atrial fibrillation	17%
Cardiomyopathy hypertensive	21%
Other	9%
Respiratory patients (n = 30)	
Chronic obstructive pulmonary disease.	37%
Respiratory infection	30%
Other (Pulmonary embolism, asthma, pneumonia, bronchitis)	33%

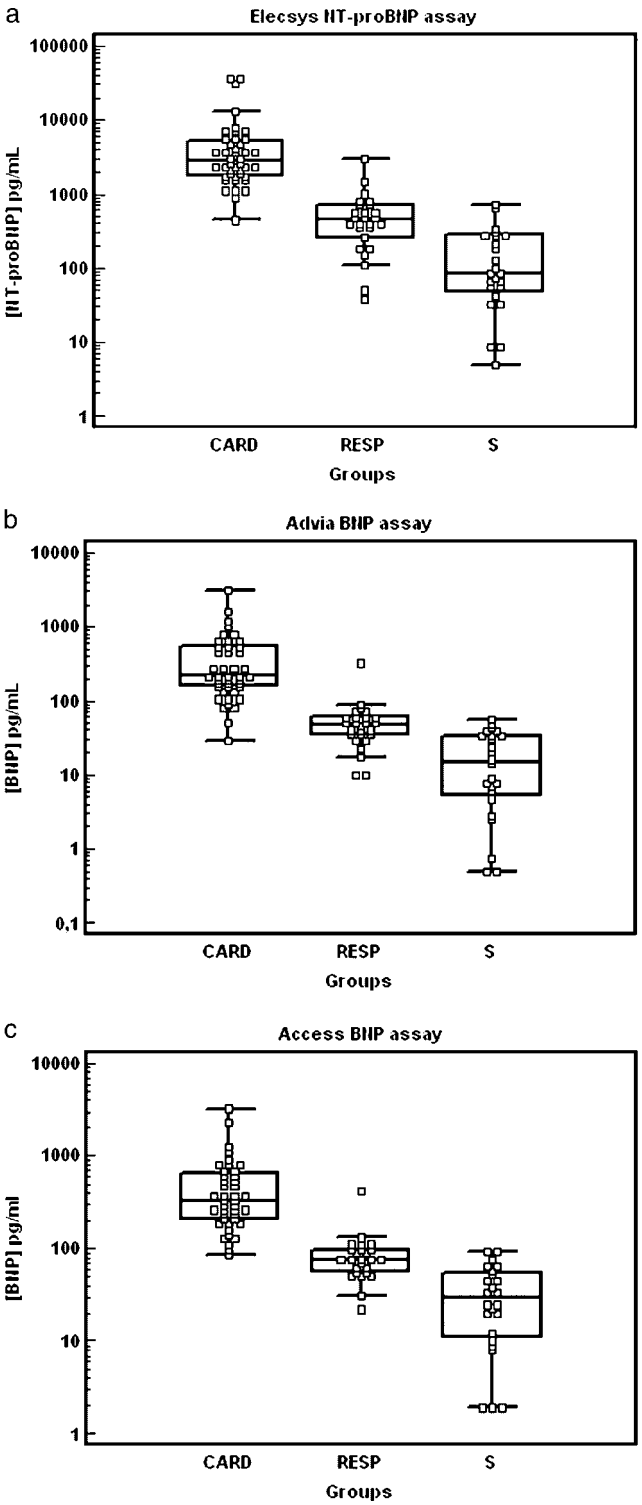

Fig. 1. Distribution of brain natriuretic peptide concentrations measured in **a:** Elecsys proBNP assay, **b:** Advia BNP assay, **c:** Access BNP assay, by patient groups. CARD, patients with cardiac dyspnea; RESP, patients with respiratory dyspnea; S, patients without cardiac nor respiratory dysfunction. The plots display the median values (lines inside boxes), 25th and 75th percentiles (lower and upper limits of boxes), and the minimum and 99th percentiles.

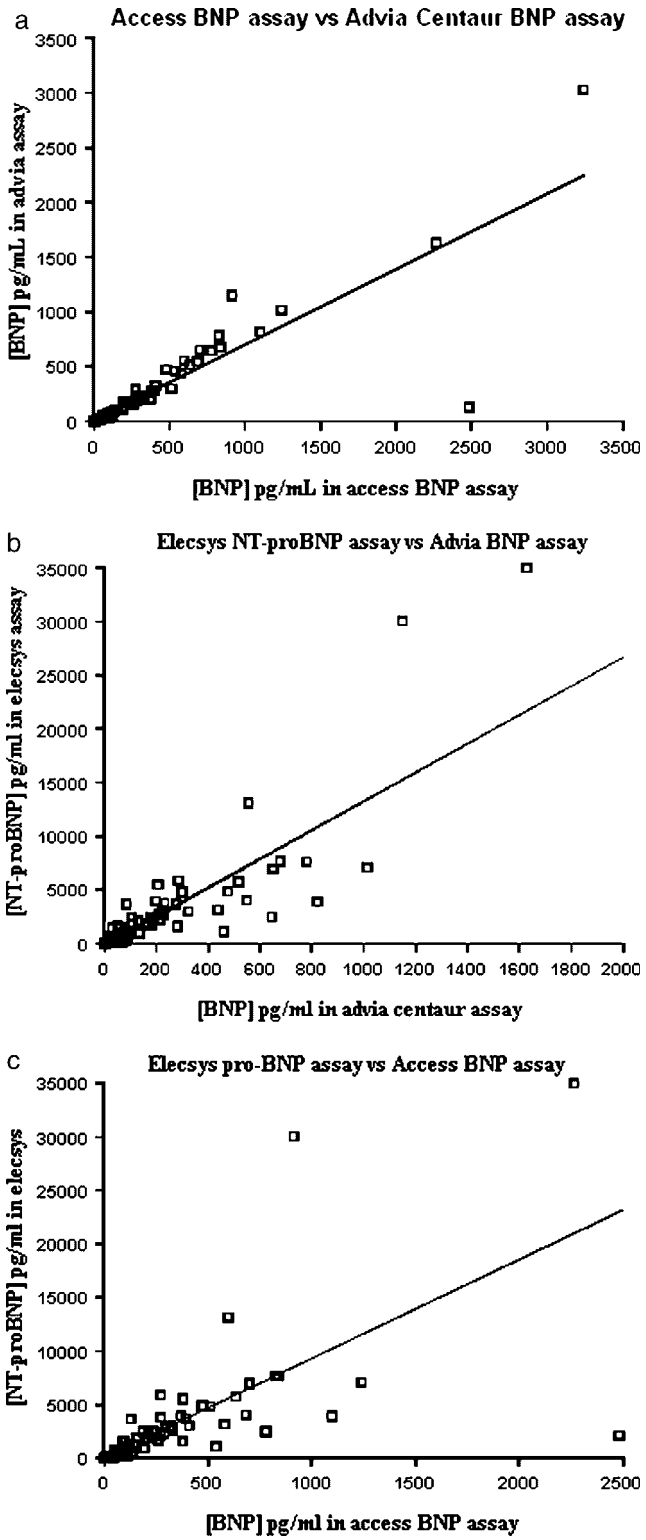

Fig. 2. Comparison of BNP and/or NT-proBNP values obtained with Access, Centaur, and Elecsys 2010 assays. **a:** Access BNP assay vs. Advia BNP assay with a linear regression: $y = -9.23 + 0.81 \times$, correlation coefficient: $r = 0.986$. **b:** Advia Centaur BNP assay vs. Elecsys NT-proBNP assay: $y = -233 + 10.1 \times$, $r = 0.876$. **c:** Access BNP assay vs. Elecsys NT-proBNP assay: $y = -79 + 12.9 \times$, $r = 0.89$.

TABLE 2. Correlation among the different assays for BNP and pro-BNP

	Slope	CI	y-intercept	CI
Access BNP vs. Advia BNP	0.81	(0.75 to 0.84)	–9.23	(–13.18 to –6.24)
Access BNP vs. Elecsys pro-BNP	10.1	(9.38 to 11.03)	–233	(–351.99 to –135.80)
Advia BNP vs. Elecsys pro-BNP	12.9	(11.49 to 15.05)	–79	(–193.44 to –23.09)

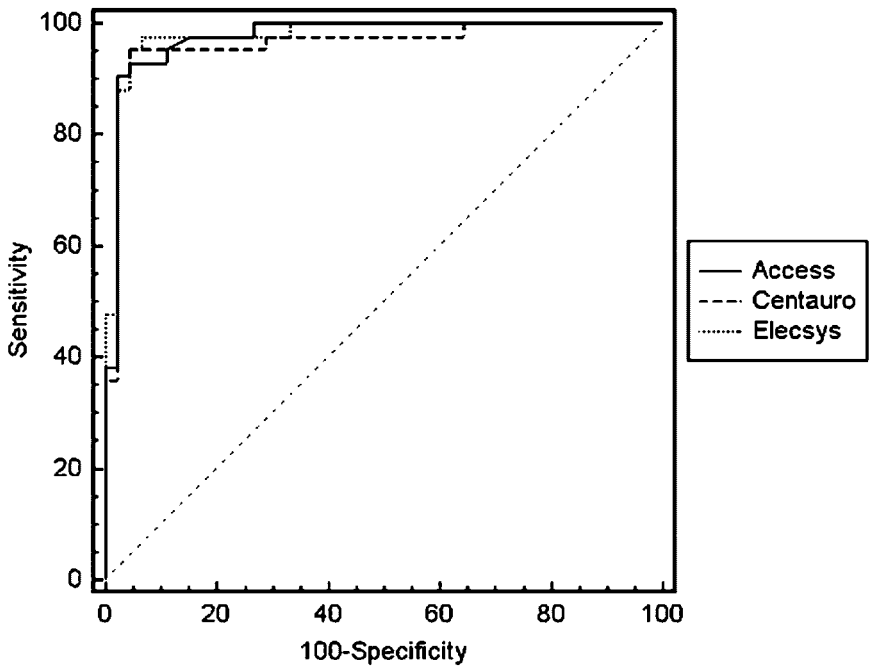

Fig. 3. ROC curve comparing sensitivity and specificity of BNP and NT-proBNP in the differential diagnosis of cardiac dyspnea. AUC's are significant ($P<0.001$).

The optimal relationship between clinical sensitivity and specificity with a 95% confidence interval was obtained with a cut-off of 116 pg/mL for Access BNP assay and at a concentration of 79 pg/mL for Advia BNP assay and 817 pg/mL for Elecsys NT-proBNP assay. The commercial specification shows a cut-off-for clinical decision of 100 pg/mL for BNP assays and a cutoff value 300 pg/mL for proBNP (men >50 years old). The diagnostic accuracy results are shown in Table 3.

DISCUSSION

B-type natriuretic peptides have emerged in the last years as important neurohormonal markers for the control of cardiovascular function (3). An early diagnosis of heart failure permits a delay in disease progression, reduces mortality, and improves the quality of life (13). Therefore, determining low concentrations of natriuretic peptides may avoid the need for more expensive and time-consuming procedures such as ultrasonography. Moreover, therapies used for congestive heart failure could be life-threatening in patients with other pathologies (e.g., chronic obstructive pulmonary disease) that have similar primary symptoms.

At present, there are different immunoassays available for the determination of natriuretic peptides (NT-proBNP and BNP). Unfortunately, the results obtained (for the same peptide) with these assays may be different (14), indicating the lack of standardization in immunoassay methods. However, there are not many studies that compare the main methods present in the market and then apply them to a certain population (15–17).

In our study, BNP assays (Advia Centaur BNP assay and Access BNP assay) were well correlated ($r>0.98$). However, both methods are subject to proportional and constant errors. Therefore, there was not a good transferability between the results obtained with them. The two assays measure the same molecule, BNP, the active form of natriuretic peptides, but the use of antibodies directed to different epitopes at the same areas accounts for the difference between them (17).

TABLE 3. Sensitivities, specificities, positive predictive value (+PV), and negative predictive value (–PV) as a function of the cutoff value obtained in this study and the recommended by the commercial specification

	Cutoff value					
	Elecsys-proBNP assay		Access BNP assay		Advia BNP assay	
	Study	Commercial	Study	Commercial	Study	Commercial
Cutoff	817	300	116	100	79	100
Sensitivity	97.7	100	92.9	95.2	95.3	86
Specificity	93.5	50	95.6	88.9	95.7	97.8
+PV	93.3	65.2	95.1	88.8	95.3	97.4
–PV	97.7	100	93.5	94.9	95.7	88.2

The lack of standardization contributes to the disparities among different assays. BNP assays correlate reasonably well ($r > 0.87$) with NT-proBNP (15).

Differential diagnosis is especially important in patients suffering coexisting respiratory and cardiac pathological conditions that are common in older patients, which often overlap or could be confused with one another. NT-proBNP and BNP concentrations found in cardiac patients with dyspnea were significantly higher than those of the respiratory group. Higher values in the respiratory group with respect to the control group could be explained by hypoxia and sympathetic overdrive originating in a secretion of natriuretic peptides.

After reviewing all of the patient information, we observed that high natriuretic peptide concentrations in initial respiratory care correspond to patients who have an associated cardiac pathology. A history of obstructive or restrictive lung disease leading to heart failure was present in four patients. Taking into consideration the idea that lung hypertension is one of the main complications of respiratory dysfunction (18), they were re-evaluated as cardiac.

It is known that natriuretic peptide concentrations are influenced by age and renal function, particularly NT-proBNP. BNP is cleared mainly from the circulation by natriuretic peptide C receptor and neutral endopeptidases, whereas NT-proBNP is cleared mainly by the kidneys (19). Therefore, BNP is less influenced by renal function and NT-proBNP could be overestimated in patients with severe renal dysfunction. We found that two patients with advance age had elevated NT-proBNP and normal BNP values, and three cardiac patients with renal dysfunction showed higher NT-proBNP than the corresponding BNP values.

Moreover, there was a discrepant value between BNP measured by Access (Triage) and Centaur assay and also with the NT-proBNP levels. This could be attributed to HAMA antibodies because the Access (Triage) BNP assay is the only one that uses murine antibodies in the development of the immunoassay.

The discrimination power between cardiac or respiratory dyspnea was comparable with the different methods assayed, although Elecsys proBNP and BNP Access assays showed the highest AUCs. To define the diagnostic cut-off for each test, we assumed the best diagnosis accuracy (the optimal diagnostic sensitivity and specificity), corresponding to a concentration of 116 pg/mL (BNP-Access), 79 pg/mL (BNP-Centaur), and 817 pg/mL (proBNP-Elecsys). These values had the greatest ability to discriminate among patients with dyspnea and only a few patients ($<7\%$) could be misdiagnosed. The manufacturers' specifications and some studies recommend the BNP assays show a decision threshold at 100 pg/mL but that did not improve the diagnosis accuracy (20).

Elecsys NT-proBNP assay is slightly superior in distinguishing cardiac dysfunction (a negative predictive value of 97.7%). For NT-proBNP, we used a different cut-off than that indicated by the commercial specification for patients >50 years old. The result obtained in this study was similar to other NT-proBNP cutoff values with the Elecsys NT-proBNPQ for patients >75 years old.

The relationship between the age and gender of patients and natriuretic peptides was not significant ($P < 0.001$). Because our study population was older (mean, 75 years old), we took only one cut-off point. For younger patients, specially for NT-proBNP, it would be necessary to establish different cut-offs values.

Finally, the situation in the emergency laboratory requires a good analytical performance and a fast and efficient response to the needs of the emergency unit. A point-of-care testing like Triage BNP could be interesting on a small unit with not so many samples, but it requires hands-on labor time. For a larger number of samples, an automatic system like Elecsys proBNP, Centaur BNP, or Access BNP assays are more suitable.

Moreover, it would be necessary to study differences in calibrator composition, specific molecular forms, and differences in antibody reactivities in order to achieve interchangeable results between assays, so that clinical confusion and differences in diagnosis may be minimized.

In conclusion, the development of different assays, standardized and easy to perform, leads to considering NT-proBNP or BNP as excellent biochemical markers (21) in the differential diagnosis of dyspnea in the emergency room. They showed good sensitivity and specificity for the diagnosis of heart failure, thereby permitting the early use of intensive therapy.

REFERENCES

1. McCullough PA, Omland T, Maisel AS. B-type natriuretic peptides: a diagnostic breakthrough for clinicians. Rev Cardiovasc Med 2003;4:72–80.
2. Mair J, Hammerer A, Puschendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. Clin Chem Lab Med 2001;39:571–588.
3. Mair J, Friedl W, Thomas S, Puschendorf B. Natriuretic peptides in assessment of left-ventricular dysfunction. Scand J Clin Lab Invest 1999;59:132–142.
4. Hall C. Essential biochemistry and physiology of (NT-pro)BNP. Eur J Heart Fail 2004;6:257–260.
5. Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides. Clin Chem 2004;50:2465–2468.
6. Cowie MR, Struthers AD, Wood DA, et al. Value of natriuretic peptides in assessment of patients with possible new heart in primary care. Lancet 1997;350:1349–1353.
7. McDonagh TA, Robb SD, Murdoch DR, et al. Biochemical detection of left-ventricular systolic dysfunction. Lancet 1998; 351:9–13.
8. Darbar D, Davidson NC, Gillespie N, et al. Diagnostic value of B-type natriuretic peptide concentrations in patients with acute myocardial infarction. Am J Cardiol 1996;78:284–287.
9. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet 2000;355:1126–1130.
10. Remme WJ, Swedberg K. Guidelines for the diagnosis and treatment of chronic heart failure. Eur Heart J 2001;22: 1527–1560.
11. McCullough, PA, Nowak RM, McCord J, et al. B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure analysis from breathing not properly (BNP) multinational study. Circulation 2002;106:416–422.
12. Mueller C, Scholer A, Laule-Kilian K, et al. Use of B-type natriuretic peptide in the evaluation and management of acute dyspnea. N Engl J Med 2004;350:647–654.
13. Davies MK, Hobbs FDR, Davis RC, et al. Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study. Lancet 2001;358:439–444.
14. Prontera C, Emdin M, Zucchelli GC, Ripoli A, Passino C, Clerico A. Analytical performance and diagnostic accuracy of a fully-automated electrochemiluminescent assay for the N-terminal fragment of the pro-peptide of brain natriuretic peptide in patients with cardiomyopathy: comparison with immunoradiometric assay methods for brain natriuretic peptide and atrial natriuretic peptide. Clin Chem Lab Med 2004;42:37–44.
15. Rawlins ML, Owen WE, Roberts WL. performance characteristics of four automated natriuretic peptide assays. Am J Clin Pathol 2005;123:439–445.
16. Yeo KTJ, Wu AHB, Apple FS, et al. Muticenter evaluation of the Roche NT-proBNP assay and comparison to the Biosite Triage BNP assay. Clin Chim Acta 2003;338:107–115.
17. Wu AHB, Packer M, Smith A, 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;50:867–873.
18. Cook DG, Shaper AG. Breathlessness, lung function and the risk of heart attack. Eur Heart J 1988;9:1215–1222.
19. Vanderheyden M, Bartunek J, Goethals M. Brain and other natriuretic peptides: molecular aspects. Eur J Heart Fail 2004;6:261–268.
20. Ala-Kopsal M, Magga J, Peuhkurinen K. Molecular heterogeneity has a major impact on the measurement of circulating N-terminal fragments of A-and B-type natriuretic peptides. Clin Chem 2004; 50:1576–1588.
21. Cowie MR. B-type natriuretic peptide testing: where are we now?. Heart 2004;90:725–726.