Head to head comparison of N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide in patients with/without left ventricular systolic dysfunction


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Abstract

Background: Human pro-B-type natriuretic peptide is cleaved into the active B-type natriuretic peptide (BNP) and the inactive fragment NT-proBNP. It is unclear if, similar to BNP, NT-proBNP can be used as a marker of impaired left ventricular (LV) ejection fraction (EF). This study evaluated the analytical performance of both assays to detect LV systolic dysfunction.

Methods: In 72 patients with various degrees of left ventricular systolic dysfunction (LVSD), blood analysis for BNP and NT-proBNP was performed prior to cardiac catheterization, using a point-of-care analyzer (Biosite) and a fully automated laboratory analyzer (Roche-Elecsys), respectively. The within-run and between-run imprecision for BNP and NT-proBNP was calculated.

Results: Both markers were able to detect impaired LV EF with the largest area under the receiver-operating-characteristic curve for NT-proBNP (NT-proBNP: 0.851 (0.747–0.924); BNP: 0.803 (0.692–0.887) 95% confidence interval; \( P = 0.07 \)). A significant correlation was observed between BNP and NT-proBNP (\( r = 0.9; P < 0.0001 \)). Estimating the within-run imprecision, the coefficient of variance for BNP was 3.14% (\( n = 20 \), mean 316 ng/L) to 3.32% (\( n = 20 \), mean 820 ng/L) and for NT-proBNP 0.9% (\( n = 20 \), mean 4390.8 ng/L) to 1.4% (\( n = 20 \), mean 225 ng/L). The between-run imprecision for NT-proBNP ranged between 2.1% (\( n = 20 \), mean 224.6 ng/L) and 2% (\( n = 20 \), mean 4391 ng/L).

Optimal discriminator values for BNP and NT-proBNP were 139 ng/L and 358 ng/L, respectively. However, adjusting the BNP cut-off value to 54 ng/L improved the negative predictive value and sensitivity of the assay.

Conclusion: Similar to BNP, NT-proBNP is a promising marker in identifying LVSD. Although both assays are reliable and have good analytical performance, their diagnostic cut-off value is dynamic and population-dependent. The slightly wider detection range and the more stable structure of NT-proBNP compared to the BNP assay suggest that NT-proBNP could play an additional role in the evaluation of patients with LV systolic dysfunction.

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Keywords: B-type natriuretic peptides; Heart failure; Cardiac markers

Introduction

Elevated B-type natriuretic peptide (BNP) levels represent a final common pathway for many cardiovascular pathological states including arrhythmias, cardiac hypertrophy, ischemia and fibrosis [1]. In addition, it is well established that cardiac secretion of BNP increases with the progression of heart failure and plasma measurement of BNP has emerged as a useful, cost-effective biomarker for diagnosis and prognosis of HF [2,3]. Finally, the diagnostic utility of BNP is complemented by its therapeutic use in guiding heart failure therapy [4].

BNP, produced in bursts by the heart, originates from a 108 amino acid precursor (pro-BNP) and is released following sustained hemodynamic load [5]. Further processing of proBNP releases a biologically active and mature 32 amino acid molecule, BNP, that corresponds to the active C-terminal

Abbreviations: BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro B-type natriuretic peptide; EDVI, left ventricular end diastolic volume index; EF, ejection fraction; PCWP, pulmonary capillary wedge pressure; LVSD, left ventricular systolic dysfunction; ICMP, Ischemic cardiomyopathy.
sequence of the human B-type natriuretic peptide precursor and a 76 amino acid N-terminal fragment (NT-proBNP) [3,6]. The biologically active BNP, the intact 108 amino acid proBNP and the remaining part of the prohormone NT-proBNP all circulate in the plasma and can be measured by immunoassay. Although recent data suggest a role for NT-proBNP in the assessment of left ventricular systolic dysfunction (LVSD) and heart failure [7], the analytical performance of the NT-proBNP assay is less clear. In addition, data evaluating whether NT-proBNP is as good as BNP to detect LV systolic dysfunction are scarce [8,9].

Currently, there are two FDA approved (United States Food and Drug Administration) commercially available assays that measure BNP and NT-proBNP. First, the Biosite Triage immunochromatographic assay uses a fluorescence immunoassay to measure BNP in whole blood and plasma specimens. Biosite uses a cut-off value of 100 ng/L as a marker of left ventricular systolic dysfunction. Second, the Roche NT-proBNP electrochemiluminescent assay utilizes the fully automated analyzer Elecsys platform and measures NT-proBNP in serum or plasma by electrochemiluminescence technology [10]. The FDA approved cut-off value for NT-proBNP to detect LV systolic dysfunction is 125 ng/L.

The aims of the present study were to compare the analytical performance of the Roche NT-proBNP assay on the Elecsys platform with the “standard” Biosite BNP assay and to compare the diagnostic accuracy of BNP and NT-proBNP for the detection of LV EF impairment by using LV angiography as the reference standard.

Methods

Study population

Seventy-two consecutive patients (mean age: 65 years, range: 28–90 years) with suspected heart disease, referred for elective diagnostic heart catheterization, were enrolled prospectively. Patients with atrial fibrillation, unstable angina, recent myocardial infarction, severe liver disease, renal insufficiency defined by a serum creatinine >176.8 μmol/L and/or estimated Glomerular Filtration Rate (eGFR) < 60 mL/min were excluded. All patients were under stable medical drug therapy with angiotensin converting enzyme inhibitors (ACE-I), beta-blockers, diuretics and spironolactone for at least 2 months. Patients with left ventricular systolic function, defined by an ejection fraction (EF) >45% and without overt signs of diastolic dysfunction (LV end diastolic pressure <12 mm Hg) were considered normal [11].

All study participants were classified according to the guidelines of the AHA/ACC for the evaluation of chronic heart failure in adults [12]. Of the 72 patients admitted for cardiac catheterization, 9 proved to be healthy with respect to the heart (normal ECG, no valvular or ischemic heart disease, normal cardiac markers). Sixteen were in HF stage A, 9 in HF stage B and 38 in HF stage C (NYHA class III, n = 20; NYHA class IV, n = 18). Patients were classified by one experienced cardiologist blinded to BNP and NT-proBNP levels (Fig. 1).

Pulmonary capillary wedge pressure was measured using a Swan-Ganz catheter whereas a 6 F pigtail diagnostic catheter was positioned in the left ventricular cavity for left ventricular pressure measurement and angiogram. The LV angiogram was imaged in the right anterior and left anterior oblique positions. Left ventricular volumes and EF were derived from the single plane angiogram using the area-length method as previously described [6].

The local ethical committee approved the study and informed consent was obtained in all patients.

Measurement of BNP and NT-proBNP

Blood, obtained in all patients prior to the procedure, was collected in plastic tubes (Sarstedt) with EDTA and lithium-heparin as anticoagulant and stored for subsequent BNP and NT-proBNP measurements. BNP was analyzed using the rapid, point-of-care (POC) Triage B-type Natriuretic Peptide test (Biosite Diagnostics Inc., San Diego, California). This test uses a fluorescence immunoassay for the quantitative determination of BNP in whole blood or plasma specimens. The BNP samples were analyzed within 4 h on EDTA whole blood as recommended by the manufacturer. The kit has a measuring range of 5 ng/L to 5000 ng/L with a coefficient of variance (CV) between 9.4% and 15.2% for the within-day imprecision and between 10.1% and 16.2% for the total imprecision.

The NT-proBNP was analyzed using the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Indiana), which uses electrochemiluminescence technology with a measuring range of 5 ng/L to 35,000 ng/L. The NT-proBNP samples were stored as lithium heparin plasma at −20°C and analyzed in batch according to the manufacturer’s recommendations [6,13]. Reproducibility on the Elecsys 2010 was determined by the
manufacturer using a modified protocol (EP-5A) of the NCCLS (National Committee for Clinical Laboratory Standards). For the total imprecision, the CV varied between 2.3% and 3.2% for human pooled serum, and between 2.2% and 2.4% using control samples. For the within-run imprecision, the CV varied between 1.8% and 2.7% for human pooled serum and 1.8% using control samples.

**Statistical analysis**

To determine reproducibility for BNP and NT-proBNP, the NCCLS (National Committee for Clinical Laboratory Standards) protocol (EP-5A) was used. Spearman’s rank correlation and Passing and Bablok regression were used where appropriate. Receiver operating characteristic (ROC) curves were constructed for NT-proBNP and BNP to detect LVSD, defined by an EF ≤45% on LV angiogram [11,13]. From these ROC curves, the optimized cut-off value for BNP and NT-proBNP was derived and using this cut-off value the diagnostic sensitivity and specificity of each assay were calculated [3,6]. Since the distribution of BNP and NT-proBNP levels was not normally distributed, all data were expressed as log-transformed values, unless otherwise specified and non-parametric tests were used. Statistical significance was defined as $P < 0.05$ (MedCalc® Statistical Software, Belgium).

**Results**

**Clinical and hemodynamic characteristics**

Clinical and hemodynamic characteristics of the study population are shown in Table 1. The study population consisted of 72 patients with a mean age of 65 years (range 28–90 years). There was a male predominance (71%) and LV EF ranged from 10 to 89% with a mean of 53%. Diagnosis of ischemic heart disease was made in 42%, valvular heart disease in 42% and idiopathic dilated cardiomyopathy in 16% of patients. Thirty-two patients had normal left ventricular function (Group A) whereas 40 patients were characterized by left ventricular systolic dysfunction (Group B).

**Plasma levels of BNP and NT-proBNP**

The BNP levels ranged from 13 to 2530 ng/L, with a mean of 309 ng/L, whereas the NT-proBNP ranged from 34 to 24,099 ng/L, with a mean of 1978 ng/L. No difference in BNP and NT-proBNP was noted between patients with ischemic heart disease, valvular heart disease and idiopathic dilated cardiomyopathy. Compared to patients with normal LV function, patients with impaired LV systolic function had significantly higher BNP ($435 \pm 458$ vs. $146 \pm 201$ ng/L; $P = 0.001$) and NT-proBNP ($3322 \pm 4697$ vs. $674 \pm 1242$ ng/L; $P = 0.002$) levels. In addition, patients with HF stage A had significantly lower BNP and NT-proBNP levels compared to HF stage B and stage C pts. Similarly, HF stage B patients were characterized by higher BNP and NT-proBNP levels compared to HF stage C pts (Fig. 2).

**BNP/NT-proBNP correlation with LV hemodynamics**

BNP as well as NT-proBNP levels showed a significant correlation (Spearman’s rank) with LV EF (Fig. 2), left ventricular indexed end diastolic volume (LV EDVI) (BNP: $r = 0.545$, $P < 0.001$; NT-proBNP: $r = 0.484$, $P < 0.001$) and pulmonary capillary wedge pressure (PCWP) (BNP: $r = 0.396$, $P = 0.009$; NT-proBNP: $r = 0.431$, $P = 0.004$).

**Analytical variation and correlation of BNP and NT-proBNP**

The coefficient of variance of BNP for the within-run imprecision ranged from 3.14% ($n = 20$, mean 316 ng/L) to 3.32% ($n = 20$, mean 820 ng/L). In contrast, the NT-proBNP assay showed a within-run and between-run imprecision with a coefficient of variance for within-run ranging from 0.9% ($n = 20$, mean 4391 ng/L) to 1.4% ($n = 20$, mean 225 ng/L), which was approximately 3 times lower than that of BNP. The

<table>
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<th>Mean ± SD</th>
<th>Range</th>
<th>Impaired LV EF ($n = 40$)</th>
<th>Normal LV EF ($n = 32$)</th>
<th>$P$ value</th>
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<tr>
<td><strong>Demographic and clinical parameters</strong></td>
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<tr>
<td>Age (years)</td>
<td>65 ± 12</td>
<td>28–90</td>
<td>61 ± 11</td>
<td>67 ± 12</td>
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<td>ICMP (%)</td>
<td>42</td>
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<td>58</td>
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<td><strong>Hemodynamic parameters</strong></td>
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<td>LVEF (%)</td>
<td>53</td>
<td>10–89</td>
<td>28 ± 11</td>
<td>72 ± 12</td>
<td>&lt;0.001</td>
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<tr>
<td>LVEDVI (mL/m²)</td>
<td>103 ± 52</td>
<td>43–296</td>
<td>132 ± 51</td>
<td>82 ± 42</td>
<td>&lt;0.001</td>
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<td>PCWP (mm Hg)</td>
<td>15 ± 9</td>
<td>3–38</td>
<td>18 ± 10</td>
<td>11 ± 5</td>
<td>0.011</td>
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<td><strong>Biochemical markers</strong></td>
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<td>BNP (ng/L)</td>
<td>309 ± 397</td>
<td>13–2530</td>
<td>435 ± 458</td>
<td>146 ± 201</td>
<td>0.001</td>
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<tr>
<td>NT-proBNP (ng/L)</td>
<td>1978 ± 3332</td>
<td>34–24099</td>
<td>3322 ± 4697</td>
<td>674 ± 1242</td>
<td>0.002</td>
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</tbody>
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LVEF = left ventricular ejection fraction, LVEDVI = left ventricular end diastolic volume index, PCWP = pulmonary capillary wedge pressure, PA = pulmonary artery pressure ($n = 72$).

Hemodynamics, BNP and NT-proBNP levels in patients with and without LV dysfunction.
between-run imprecision for NT-proBNP ranged between 2.1% ($n = 20$, mean 225 ng/L) and 2% ($n = 20$, mean 4391 ng/L). Both BNP and NT-proBNP levels showed significant correlation, with the NT-proBNP values approximately 6-fold higher than the corresponding BNP value ($r = 0.90$; $P < 0.0001$). Linear regression analysis showed a slope of 6.68 with a negative intercept of 139 ng/L.

**Diagnostic performance**

The ROC analysis for discrimination of LVSD showed a similar diagnostic performance for NT-proBNP compared to BNP with an area under the curve (95% confidence interval) of 0.851 (0.747–0.924) and 0.803 (0.692–0.887), respectively ($P = 0.07$) (Fig. 3). According to the ROC analysis, the best discriminator value of NT-proBNP with the highest sum of specificity and sensitivity was 358 ng/L. When this cut-off value was used for NT-proBNP, all but 19 patients were correctly identified with a sensitivity and specificity of 100 and 61%, respectively. By adjusting the discriminator value to the FDA proposed cut-off value of 125 ng/L, the diagnostic accuracy of the test decreased significantly with a rise in the number of false positive and negative results.

The value of BNP with the best predictive accuracy (the highest sum of sensitivity and specificity) was 139 ng/L. The number of erroneous classifications was significantly higher with the BNP assay as compared to the NT-proBNP assay. Since
one of the goals of this test is to exclude LVSD, its negative predictive value can be increased up to 100% by lowering the cut-off value from 139 ng/L to 29.3 ng/L. However, when adjusting the discriminator to this low value, the number of false positive cases \((n = 34)\) increased even more (specificity 14% and positive predictive value 47%) thereby allowing unnecessary and costly exams (Table 2). By slightly adjusting the cut-off value to 54 ng/L, the number of false positive results decreased without affecting sensitivity too much (96.8%; 95% CI (83.2–99.5)) and specificity (39%; 95% CI (24.2–55.5)) and without improving the negative predictive value (Table 2). In addition, this value restored the ancient BNP cut-off (50 ng/L) (sensitivity 96.8, specificity 39, positive predictive value 54.5 and negative predictive value 94.1) introduced by the manufacturer and confirmed by Clerico et al. (50 ng/L) [14] and corresponds closely with the diagnostic cut-off for NT-proBNP of 358 ng/L (regression \(Y = 6.68X–139.09\); Table 2).

**Discussion**

This study demonstrates that the NT-proBNP assay has a good analytical performance with a precision limit similar to the Biosite BNP assay. Both peptides are significantly correlated and are related to markers of left ventricular contractile function. In addition, this study demonstrates that, dependent upon the population studied, both FDA approved BNP and NT-proBNP cut-off values have to be readjusted in order to optimize their sensitivity and specificity. Finally, the results confirm that NT-proBNP is a helpful marker for the detection of an impaired EF.

**Analytical performance of BNP and NT-proBNP assay**

Recent studies have demonstrated that similar to BNP, NT-proBNP levels can predict the presence of left ventricular dysfunction [15], guide heart failure therapy [4] and play a role in risk stratification of patients with acute coronary syndromes [7]. One of the advantages of measuring NT-proBNP, when compared to BNP, is that by being the inactive cleavage product of proBNP, it demonstrates a longer biological half-life and better stability, both in circulating blood and after sampling [16]. This higher stability is reflected in the NT-proBNP assay which appears robust and has a high CV with a wide measuring range. In addition, the assay is highly specific for the NT-proBNP with less than 0.01% cross-reactivity with the BNP-32 bioactive molecule [17]. The three times higher CV of BNP compared to NT-proBNP is not unexpected for an assay initially developed as a point of care device. Although newer adaptations of the BNP assay on the Bayer Adura Centaur automated analyzer demonstrated precision comparable to the one obtained for the NT-proBNP assay [18], we could not confirm these findings for the Biosite triage system. This may be important, especially if there are delays in sample analysis and finally might make NT-proBNP a better and more reliable marker than BNP for the detection and evaluation of heart failure [19].

**BNP and NT-proBNP as a marker of impaired LV EF**

Both BNP and NT-proBNP were closely related to hemodynamic and angiographic parameters of left ventricular systolic dysfunction, corroborating previous observations that wall stress is a major stimulus for BNP production [3,6]. Although a significant correlation was noted between BNP and NT-proBNP, a small subgroup of patients with elevated NT-proBNP values had BNP levels below the FDA cut-off value of 100 ng/L. This discrepancy has been attributed to age-dependent differences in proBNP cleavage and NT-proBNP clearance [16] as well as to the longer biological half-life of NT-proBNP compared to BNP [19]. In addition, the discrepancy between the relatively higher NT-proBNP compared to BNP levels may be explained by trimming of the endogenous BNP by aminopeptidases [20]. Of note, recent work has demonstrated that human synthetic BNP is degraded when incubated in whole blood into a dominant BNP form lacking the 2 N-terminal amino acids [21]. Although this N-terminal region does not seem to be critical for receptor binding and biological activity, the enzymatic cleavage at the aminoterminal may be critical when choosing epitopes for antibody production and immunoassays.

**Population-based reference value of BNP and NT-proBNP**

In our study population, the FDA approved cut-off value for BNP and NT-proBNP, which was chosen to exclude false negative results when screening a general population, appears to be too low. This inaccuracy of the FDA cut-off value can be explained by the difference in the study population. Where FDA cut-off values are aimed at detecting LV dysfunction in the general population, our study looked for a discriminator value in

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**Table 2**

Diagnostic performance of BNP and NT-proBNP to detect LVSD when using the optimal discriminator values of 54.2 and 358 ng/L as well as when applying the FDA approved cut-off values of 100 and 125 ng/L, respectively \((n = 72)\)

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<th>BNP</th>
<th>NT-proBNP</th>
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<td></td>
<td>ROC analysis, 139 ng/L</td>
<td>ROC analysis with 100% NPV, 29.3 ng/L</td>
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<tr>
<td>Sensitivity</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>71</td>
<td>14</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>68</td>
<td>47</td>
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<tr>
<td>Negative predictive value</td>
<td>85</td>
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a selected group of patients in whom cardiovascular disease was likely. Readjusting the cut-off value of BNP and NT-proBNP to 54 and 358 ng/L, respectively, increased the negative predictive value of both assays and resulted in an improved sensitivity to detect LV systolic dysfunction. This was achieved without major concessions in the specificity of both assays. This urge of using dynamic cut-off values corroborates previous reports [17,22] and highlights the importance of using appropriate population-based reference ranges (cut-off points) for NT-proBNP and BNP in the assessment of CHF [14,23].

Conclusion

We demonstrate that both assays are robust and are helpful in the evaluation of patients with left ventricular systolic dysfunction. Despite a trend towards better precision for the NT-proBNP, no statistically significant differences in diagnostic performance were noted between both assays. However, the slightly wider detection range and the more stable structure of NT-proBNP may favor its use over BNP in the evaluation of patients with left ventricular systolic dysfunction. Prospective clinical studies using both BNP and NT-proBNP are needed to determine which marker is best in a particular clinical setting.

References