Congestive Heart Failure

**A rapid B-type natriuretic peptide assay accurately diagnoses left ventricular dysfunction and heart failure: A multicenter evaluation**

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**Background** B-Type natriuretic peptide (BNP), a protein released from the left ventricle in response to volume expansion and pressure overload, has emerged as the first whole blood marker for the identification of individuals with congestive heart failure (CHF).

**Objective** The purpose of this study was to assess the performance of a point-of-care assay to diagnose and evaluate the severity of CHF on the basis of the New York Heart Association (NYHA) classification system.

**Methods** Through a prospective, multicenter trial, whole blood samples were collected from a total of 1050 inpatients, outpatients, and healthy control patients. Participants were divided into subgroups for BNP analysis: patients without cardiovascular CHF (n = 473), patients with hypertension and no cardiovascular disease (n = 168), NYHA class I CHF (n = 73), class II CHF (n = 135), class III CHF (n = 141), and class IV CHF (n = 60).

**Results** Circulating BNP concentrations determined from the bedside assay increased with CHF severity, as determined by the NYHA classification system, but were only statistically significant (P < .001) between individuals with and without CHF. Individuals without CHF had a median BNP concentration of 9.29 pg/mL. Median BNP values, with their corresponding interquartile ranges, for NYHA classification I through IV were 83.1 pg/mL (49.4-137 pg/mL), 235 pg/mL (137-391 pg/mL), 459 pg/mL (200-871 pg/mL), and 1119 pg/mL (728-1300 pg/mL), respectively. With the use of a decision threshold of 100 pg/mL, the assay demonstrated 82% sensitivity and 99% specificity for distinguishing control patients and patients with CHF.

**Conclusions** BNP concentrations obtained from whole blood samples are useful in the diagnosis of CHF and staging the severity of the disease. (Am Heart J 2002;144:834-9.)

Congestive heart failure (CHF) is a common health problem in the United States, with 400,000 to 700,000 new cases each year. The disease occurs in 1.5% of the population, and its prevalence increases to 20% in individuals aged >70 years.1 The key to treatment of this disease is early detection and intervention because administration of therapeutic agents such as angiotensin-converting enzyme (ACE) inhibitors and β-adrenergic receptor blockers have been shown to improve survival.2 Until the recent discovery of B-type natriuretic peptide (BNP), a novel biomarker has not been available to aid physicians in caring for these patients.

BNP is a component of the natriuretic peptide system that is stimulated when insufficient amounts of blood are pumped to the body. In this system, the heart secretes 2 different peptides, atrial natriuretic peptide (ANP) and BNP, in response to ventricular dysfunction. ANP is produced in the atria and is released in response to increased atrial wall tension. BNP, also commonly known as brain natriuretic peptide, was first isolated from porcine brains and later from hearts of pigs and rats.3,4 It is a 32-amino acid protein that is released from the ventricles in response to increased ventricular overload. Of these 2 peptides, BNP has...
emerged as a superior marker for CHF and left-ventricular dysfunction because it is rapidly activated at the gene level in high concentrations, is more stable than ANP, and is more sensitive to left ventricular dysfunction.5-9

The increased awareness and clinical significance of BNP in patients with CHF has warranted the development of the first commercial point-of-care assay. This initial study was conducted to validate the Triage BNP assay (Biosite Diagnostics, San Diego, Calif) in a clinical setting. To fulfill this goal, circulating BNP concentrations were analyzed in both CHF and non-CHF populations, and a diagnostic reference range was determined.

Methods

Patient population

The patient protocol was reviewed and approved by the Institutional Review Boards at Hartford Hospital, University of Connecticut Health Center, Albany Medical Center, San Diego VA Medical Center, and University of Maryland. In this study, BNP concentrations were measured for both a CHF and a non-CHF control group. The CHF group consisted of 409 patients in whom the diagnosis of CHF and New York Heart Association (NYHA) classification was performed by a physician on the basis of clinical history and physical examination. With the use of this criteria, 73 NYHA class I, 135 class II, 141 class III, and 60 class IV patients were enrolled in the study on oral or written consent. In this population, 28% were idiopathic, 43% were ischemic, 19% were other causes such as hypertension or alcoholism, and 10% were unknown. In addition, the ejection fraction was noted if the test was performed within 12 months of physical examination. Systolic dysfunction was defined as an ejection fraction (EF) ≥45% and diastolic dysfunction was defined as an EF >45%. By use of this criteria, there were 251 patients with systolic dysfunction and 71 patients with diastolic dysfunction.

The control group consisted of 473 individuals who had no evidence of heart failure on examination or any abnormality of left ventricular function if an echocardiogram was performed. Patients excluded were those with end-stage kidney failure or acute myocardial infarction. In addition, patients who had hypertension (n = 168) but no evidence of CHF were analyzed to determine if BNP levels were elevated. Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg.

Triage BNP assay

All samples were collected by venipuncture into EDTA tubes. The blood samples were kept at room temperature and analyzed within 4 hours of the draw time. Before analysis, each tube was inverted several times to ensure homogeneity. The whole blood was then analyzed in triplicate with the Triage BNP assay. This sandwich immunoassay consists of a disposable device to which 250 mL of EDTA-anticoagulated whole blood is added. A Triage Meter was then used to measure the BNP concentration by detecting a fluorescent signal that reflects the BNP concentration of the sample. The test is self-processing, with results available within 15 minutes. The detection range of the BNP assay is 5 to 1300 pg/mL. Samples cannot be diluted to obtain results exceeding 1300 pg/mL.

Evaluation of analytical performance

Assay precision. Intra-assay and interassay imprecision was determined on the basis of the analysis of variance model by testing 3 control samples (28.8 pg/mL, 584 pg/mL, 1180 pg/mL) that spanned the range of the standard curve. The BNP concentration of these samples was measured 10 times per day and repeated for 10 days.

BNP stability. BNP has been found to be stable at room temperature for 24 hours, or in up to 30°C for 12 hours in the presence and absence of aprotinin, on the condition that brain natriuretic peptide is assayed within 1 month (frozen at −20°C) after blood collection. Additionally, the presence of aprotinin prevents brain natriuretic peptide degradation in samples preserved for >1 month at −20°C before assay.

Whole blood versus plasma. The measurement of BNP in whole blood versus plasma was compared for 100 random patients with CHF. For these samples, 250 mL of whole blood was added to the Triage device and the remainder was immediately centrifuged at 2500 RPM for 10 minutes to separate plasma from cells. On completion, the plasma was immediately analyzed by the whole blood protocol.

Statistical analysis

All results are expressed in median values as well as with the corresponding quartile interval (25th to 75th percentiles). Probability values were calculated by Mann Whitney comparison for CHF versus normal individuals, normal versus hypertension, men versus women, and different age groups to determine if results were statistically significant. Receiver operator characteristic (ROC) curves were performed with the SPSS program (SPSS Inc, Chicago, Ill). These curves plot sensitivity versus 1-specificity for both control patients and patients with CHF and are used to evaluate the sensitivity and specificity of the assay at different diagnostic thresholds. The area under the curve was reported with 95% CIs. BNP concentrations at or above the diagnostic threshold were considered positive, whereas values below the threshold were considered negative. Assay precision studies were analyzed by means of the analysis of variance model, and the difference between 2 measurements was considered significant if it was >2 SD.

Results

Analytical performance of the triage BNP assay

Precision. The coefficient of variation (CV) for intra-assay precision was 9.5% at 28.8 pg/mL, 12.8% at 584 pg/mL, and 13.9% at 1180 pg/mL. The CV for interassay variation was 10% at 28.8 pg/mL, 12.4% at 584 pg/mL, and 14.8% at 1180 pg/mL. BNP concentrations measured in plasma and whole blood were not significantly different (r = 0.925x + 13.439, r = 0.9878).
BNP stability. BNP stability varied between room temperature and 4°C. All of the samples were stable up to 8 hours at 4°C. At room temperature, however, 3 samples were stable for 8 hours and 3 samples were stable for 4 hours. Therefore, the minimum stability of BNP at room temperature was determined to be 4 hours.

Clinical evaluation

Circulating BNP concentrations were not consistent throughout the control population and support previous findings (Figure 1).11,12 Median BNP concentrations were higher in men than in women and increased with age. The median BNP concentration and corresponding quartile for noncardiac men was 5.47 pg/mL (5.0 to 12.8 pg/mL) and 12.8 pg/mL (5.82 to 30.6 pg/mL) for noncardiac women ($P < .001$). Median BNP concentrations also increased with age for both men and women. When compared with individuals (aged 35 years), the gradual increase became significant for individuals aged 55 to 64 years ($P < .01$) and further increased for the next decade ($P < .001$). The slight increase observed between adjacent decades, however, was not significant. In the hypertensive subgroup, BNP concentrations for the entire age range of hypertensive patients did not show any statistical significance from the age-matched control population (Table I). Thus the patients from the non-CHF group and the hypertensive subgroup were pooled together to constitute the control group for further analysis.

The circulating BNP concentration was also measured for patients with CHF in each of the 4 NYHA classes (Figure 2). BNP concentration increased with CHF severity, determined by NYHA classification, but could not predict the different NYHA classes because of considerable overlapping of CIs. The median BNP values and corresponding quartiles were 83.1 pg/mL (49.4-137 pg/mL), 235 pg/mL (137-391 pg/mL), 459 pg/mL (200-871 pg/mL), and 1119 pg/mL (728-1300 pg/mL) for classes I through IV, respectively. The cluster of points at 1300 pg/mL occurred because it is the maximal detectable concentration of BNP. There was also a negative linear relation between BNP and systolic ejection fraction ($r = -0.352$, $P < .001$).

Diagnostic specificity for detection of CHF

The ROC curve in Figure 3 plots the sensitivity versus 1-specificity for BNP levels in distinguishing between normal patients and all patients with CHF (NYHA class I through IV). The area under the curve represents the overall accuracy of the test and was found to be 0.93 ($P < .001$). At a cut-point of 100 pg/mL (elbow of the curve and FDA-approved diagnostic cutoff), the specificity was 97%, with a sensitivity of 82%. Figure 4 shows a box-and-whisker plot of the BNP levels in patients with CHF (NYHA class I through IV) compared with control patients ($P < .001$). ROC curves were plotted for BNP versus each NYHA class (Table II). In our population, in each NYHA class, BNP was able to successfully separate normal patients from patients with each class of CHF. NYHA classes demonstrated that the area under the curve and corresponding 95th percent CIs increased with disease severity as determined by the NYHA classification system (Table II). The highest assay sensitivity and specificity was for the ROC curve between NYHA class IV and non-CHF patients and lowest was for the ROC curve between NYHA class I (asymptomatic CHF) and non-CHF patients.

Discussion

Although major advances in understanding the pathophysiology of CHF have resulted in treatments...
that lead to symptomatic improvement and longer life, CHF remains a major clinical challenge. Not only do we have difficulty making the diagnosis of heart failure, but it is also difficult to assess the results of treatment, both in the hospital and the outpatient setting.

Direct costs of heart failure exceed $38 billion dollars in the United States, over 5% of the total health care costs, suggesting that we must continue to search for measures that will improve diagnostic and therapeutic outcomes, while continuing the effort for a better understanding of its pathophysiology.\(^\text{13-17}\)

The Triage BNP assay is the first whole blood test to aid in the diagnosis of patients with CHF. Previous studies have validated the Triage BNP assay and concluded that this immediate response assay has a relatively close correlation \((r = 0.93)\) with radioimmunoassay platforms.\(^\text{18,19}\) Although the present test has slightly higher intra-assay \((11.8\% \text{ vs } 8.6\%)\) and interas-

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**Figure 2**

Box-and-whisker plot showing median and 25th and 75th percentile levels of BNP concentrations for women (dots), men (gray), and men and women combined (black) as disease severity increases. Vertical line without dot represents range from 10% to 90%. Circles represent outliers that are <1.5% of the 75th CI; asterisks are those >1.5% of 75th CI. Cluster of points occurs at 1300 pg/mL because this is the maximum detectable concentration.

**Figure 3**

ROC curve for normal versus CHF BNP values (NYHA classes I-IV). Area under the curve = 0.971 [0.96-0.99], \(P < .001\)

**Figure 4**

Box-and-whisker plot shows range and 25th percentile/median/75th percentile box for BNP and control groups. Vertical line without dot represents range from 10% to 90% & represent outliers that are <1.5% of the 75th CI; asterisks are those >1.5% of 75th CI. Dashed line is the diagnostic threshold of 100 pg/mL.

**Table II.** BNP assay becomes more specific with disease severity

<table>
<thead>
<tr>
<th>NYHA class</th>
<th>AUC</th>
<th>Standard error</th>
<th>AUC 95th CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>0.934</td>
<td>0.018</td>
<td>0.898-0.970</td>
</tr>
<tr>
<td>Class II</td>
<td>0.957</td>
<td>0.014</td>
<td>0.930-0.984</td>
</tr>
<tr>
<td>Class III</td>
<td>0.990</td>
<td>0.003</td>
<td>0.984-0.997</td>
</tr>
<tr>
<td>Class IV</td>
<td>1.000</td>
<td>0.000</td>
<td>1.000-1.0000</td>
</tr>
<tr>
<td>Class I-IV</td>
<td>0.971</td>
<td>0.006</td>
<td>0.960-0.983</td>
</tr>
</tbody>
</table>

AUC, Area under the curve.
say (12.4% vs 6.4%) coefficients of variation, this increase is inherent in point-of-care testing and will not significantly alter clinical BNP results as shown through this multicenter clinical trial.

Although NYHA classification correlates with clinical symptoms as well as death for patients with CHF, the fact that such a subjective classification is still the major means to stage CHF remains a concern. Because BNP concentrations correlate to elevated end-diastolic pressure, which correlate closely to the dyspnea, a chief symptom of CHF, it is not surprising that BNP concentrations increased as NYHA class progressed. However, there was a pronounced overlap in BNP concentrations between the NYHA classification groups. This overlap may have represented some variability of BNP concentrations with disease state, but is also due to the subjectivity of the NYHA classification system. As observed in clinical practice, each NYHA group also contained patients with diastolic and systolic dysfunction. The BNP relation with the NYHA classification system and the overlap between classes, however, was consistent with previous studies performed on plasma samples. Direct comparison of BNP concentration observed in this study to previous studies, however, could not be performed because there is no established standard for BNP assays.

The relation of BNP concentration to CHF severity has also been noted by analytical parameters determined from echocardiography and nuclear imaging. Results of these studies demonstrate a negative, linear relation between BNP concentration and left ventricular ejection fraction (LVEF) and a positive linear relation between BNP and left ventricular end-diastolic pressure (LVEDP), pulmonary capillary wedge pressure (PCWP), and LV hypertrophy. The significant linear relation of BNP to LVEF was also observed in this study. The other parameters were not analyzed because they were not recorded as part of the clinical protocol. In another study, Yoshimura et al analyzed the specificity of BNP more critically by comparing PCWP, LVEDP, and LVEF with BNP concentration in patients with dilated cardiomyopathy and mitral valve stenosis. There was a positive linear correlation between PCWP or LVEDP and a negative linear correlation between BNP level and LVEF in the cardiomyopathy group and not in the mitral valve stenosis group. Thus these results further support the specificity of BNP for left ventricular dysfunction.

It is not unexpected that BNP concentrations are not uniform across the control population. BNP concentrations increase with age, as the left ventricle appears to stiffen over time, offering a likely stimulus to BNP production. Women with and without CHF tended to have somewhat higher BNP concentrations than did men of the same age group. Although the reason is unknown, it is possible that aging women have stiffer left ventricles than aged-matched men. There are no significant differences in BNP concentrations between patients with hypertension. BNP values may be limited in the presence of kidney failure. In the current study, we excluded patients with significant kidney failure because BNP is increased in late (predialysis) stages of kidney failure and in virtually every patient on dialysis. Part of this increase may be secondary to fluid overload. Evidence supporting this theory is that post-dialysis measurements indicate significant drops in BNP concentrations, although not back to normal levels.

The current data suggest that a BNP cut-point of 100 pg/mL allows for the increased concentrations observed with advancing age and provides an excellent ability to discriminate CHF from non-CHF subjects. The identification of clinical CHF (NYHA class II-IV) by this diagnostic threshold was especially beneficial because the sensitivity was 82% for patients with moderate CHF and up to >99% for patients with NYHA class IV CHF. This threshold is in agreement with Dao et al, who used the BNP point-of-care BNP assay for 250 patients who were examined in the emergency department for acute dyspnea. BNP concentrations <80 pg/mL had a negative predictive value of 98% for CHF.

As with all biomarker tests, relying on a single cutoff value may limit the test utility. Using the cutoff of 100 pg/mL may have some limitations in early left ventricular dysfunction, since it was only 51% sensitive in separating non-CHF patients from asymptomatic NYHA class I patients. Thus, it is possible that both a high and a low cutoff may be desired; a high one (probably ~100 pg/mL) for its specificity and positive predictive value, and a low value (40-60 pg/mL) for its high sensitivity and negative predictive value. Lower cutoffs have been especially valuable in their negative predictive value for screening asymptomatic patients with abnormal ventricular function. In particular, the use of age-specific reference intervals, which are lower than the 100 pg/mL cutoff, would also increase clinical specificity.

Although the current rapid assay has only been approved for diagnosis, it is quite possible that measuring BNP concentrations might also be an effective way to improve the inpatient treatment of patients admitted with decompensated CHF. BNP concentrations at the time of hospital discharge correlate to subsequent readmission and death. BNP concentrations also drop rapidly with effective CHF treatment. For example, BNP concentration decreased by about 35 pg/mL per hour as wedge pressures was lowered by vasodilator therapy. The fact that this drop correlates with the drop in wedge pressure might also allow BNP concentrations to help tailor treatment of patients with hemodynamic monitoring. Finally, the role of BNP in the
outpatient cardiac or primary care clinic may be critically important in referring patients for echocardiography, the titration of therapies, and to assess the state of neurohormonal compensation of the patient.29

Thus the Triage BNP assay is the first point-of-care BNP assay available to aid in the diagnosis of CHF. With the appropriate cutoffs, a high degree of diagnostic sensitivity and specificity can be achieved. The rapid, point-of-care assay should prove useful in the urgent care setting, the intensive care unit, as well as in the outpatient primary care or cardiology clinic.

References