New Frontiers in Cardiovascular Management
Clinical Experiences and State-of-the-art Research
on N-terminal Pro-brain Natriuretic Peptide (NT-proBNP)

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A Report from the 1st International Symposium on NT-proBNP
May 16-17, 2003 Lisbon, Portugal

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N-terminal pro-brain natriuretic peptide (NT-proBNP) represents the N-terminal fragment of proBNP, the high molecular weight precursor of biologically active BNP. NT-proBNP circulates at high concentrations in plasma, has a relatively long half-life, is stable in whole blood, and can easily be detected and quantified by immunometric assays. These properties make NT-proBNP a potentially useful marker for a variety of cardiovascular disorders, particularly heart failure and acute coronary syndrome (ACS). This issue of Cardiology Scientific Update reports on state-of-the-art research into NT-proBNP, including potential clinical applications. Because of the rapid development in this field, a substantial portion of the material reviewed in this Update has not yet been published and these findings should therefore be considered preliminary.

Basic physiology and analytical aspects

The mammalian natriuretic peptide system includes atrial (ANP), brain or B-type (BNP), and C-type natriuretic peptide (CNP). Brain natriuretic peptide (BNP) is a 32 amino acid cardiac natriuretic peptide that was originally isolated from porcine brain tissue. The human BNP gene is located on chromosome 1 and encodes the prohormone proBNP. As shown in Figure 1, the intact 108 amino acid proBNP (the prohormone), the biologically active 32 amino acid BNP, as well as the remaining 76 amino acid N-terminal part of the prohormone (NT-proBNP), all circulate in the blood and can be readily measured by immunoassay. In contrast to ANP-related peptides – ANP and the N-terminal portion of the ANP prohormone (NT-proANP) – that originate mainly from atrial tissue, BNP-related peptides are produced to a large extent in ventricular myocytes. Ventricular BNP and NT-proBNP are strongly upregulated in heart failure and locally in the area surrounding a myocardial infarction (MI). BNP is cleared from plasma by binding to the natriuretic clearance receptor (type C), but unlike ANP, it seems relatively resistant to proteolysis by neutral endopeptidase (NEP24.11). Little is known about the clearance mechanisms of proBNP and NT-proBNP. While plasma levels of NT-proBNP and BNP are approximately equal in normal subjects, the NT-proBNP plasma level is 2-10 times higher than the BNP level in patients with heart failure (HF). This property of NT-proBNP makes it a potentially useful marker in clinical conditions characterized by increased secretion of natriuretic peptides.

Analysis of plasma NT-proBNP concentrations

The original measurement of NT-proBNP involved a radioimmunoassay (RIA) using rabbit antiserum to N-terminal proBNP (amino acid 1-31), or human N-terminal proBNP fragment (amino acid 1-21). One of the currently commercially available assays for measuring NT-proBNP is the Elecsys® proBNP assay (Roche Diagnostic GmbH, Manheim, Germany). This assay is based on two polyclonal antibodies directed at two epitopes – residues 1-21 and 39-50 of the NT-proBNP molecule. Analytical performance is assessed as part of a multicentre evaluation using three serum pools. The single-site, within-run, percent coefficient of variation (CV), ranged from 0.7 to 1.6, depending on platform. Between-run percent CV ranged from 4.4 to 5.3. In the multicentre evaluation, within-run percent CV was 1.0-2.5. Functional sensitivity was <50 pg/ml with a measuring range up to 35,000 pg/ml.
No interference was observed with hemolyzed, icteric, and lipemic sera. Serum and heparin plasma samples demonstrated good agreement, but lower values were seen in EDTA plasma. Samples were stable for 7 days at room temperature, for 21 days at 4°C, and for 5 freeze-thaw cycles. From a population of 1205 healthy subjects (671 males, 534 females), who were screened normal by echocardiography and symptom questionnaire, the median value for males aged 45-54 was 10.5 pg/ml. Levels increased with age and were higher in women. As expected, NT-proBNP values were approximately 5 times greater than those of BNP measured by point-of-care assay Triage® (Biosite, San Diego, CA).

The higher molecular weight of NT-proBNP implies, besides better detection, greater stability in the circulation than BNP. In this regard, the biologic variability of BNP and NT-proBNP measured by different methods was recently assessed in healthy subjects over an 8-week period. BNP was measured by two manual assays, namely Triage® and Shionoria® (CIS, France), and one automated assay, Centaur® (Bayer, Leverkusen, Germany), whereas NT-proBNP was measured by one automated assay, namely Elecsys. Data for analytical variability (CVA, %) and intra-individual variability (CVI, %) are shown in Table 1. Both the CVA and CVI were higher for BNP measured by the manual and automated assays, which suggested that BNP is more variable on a day-to-day basis. This, in turn, is likely related to differences in the in vivo half-lives (20 minutes for BNP, 1-2 hours for NT-proBNP). These data suggest that NT-proBNP is potentially more useful than BNP for monitoring disease status and response to intervention.

Clinical thresholds (cut-off points) for NT-proBNP vary with geographic regions. For the Elecsys proBNP assay in Europe, health authorities request cut-off points that are based on age and gender. In European countries, the cut-off for abnormal values for male and female are 84 and 135 pg/ml, respectively, for age <50 years, and 194 and 222 pg/ml, respectively, for age 50-65 years. In the United States and Canada, the Food and Drug Administration requires only age separation. There, the cut-off for both male and female is 125 pg/ml for age <75 years and 450 pg/ml for age ≥75 years.

Because the studies reviewed here have utilized different assay methods with antibodies directed to different epitopes, and therefore, carry different conversion factors, the units of NT-proBNP reported in this Cardiology Scientific Update will therefore be the same as the ones reported by the investigators of the individual studies, with no attempt to convert to a standardized unit. However, pg/ml will be the standard measuring unit for NT-proBNP.

### NT-proBNP in LV dysfunction and heart failure

The European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of heart failure (HF) have recognized the clinical utility of natriuretic peptides in the diagnosis of HF in the following statement:\(^\text{10}\)

- These (natriuretic) peptides may be most useful clinically as a “rule out” test due to their consistent and very high predictive values
- Primary care patients, in particular those suspected of having HF, can be selected for further investigation by echocardiography or other tests of cardiac function on the basis of having elevated plasma concentrations of a natriuretic peptide
- In patients with normal concentrations, other causes of dyspnea and associated symptoms should be considered
- The added value of natriuretic peptides in this situation has yet to be determined.

The ESC has recently appointed a Task Force to update these guidelines and further define the role of BNP, including that of NT-proBNP. With the increasing use of BNP and NT-proBNP, the EuroHeart Failure Survey Program,\(^\text{11}\) a program examining the quality of care for HF patients in Europe, has started to collect data on these peptides as part of their survey. The following sections will provide an update on the data regarding NT-proBNP in left ventricular (LV) dysfunction and HF.

### NT-proBNP as a diagnostic screening tool to differentiate between patients with normal and impaired LV function

To evaluate whether NT-proBNP measurements can be used to differentiate between normal and reduced LV ejection fraction in unselected patients, plasma was obtained from 2230 admissions to a general city hospital in Copenhagen, Denmark, over a 10-month period.\(^\text{12}\) Patients also had echocardiography to determine LV function. The performance characteristics of NT-proBNP are shown in Table 2. A raised NT-proBNP level of ≥357 pmol/L identified patients with an LV ejection fraction of ≤40% (n=157), with high specificity and negative predictive value. Plasma NT-proBNP levels increased with age. The relationships between NT-proBNP and age, gender, serum creatinine, and LV ejection fraction were examined in a multivariate regression analysis. The combination of age and NT-proBNP was most important in predicting LV ejection fraction ≤40%, while gender and serum creatinine had only a minor impact. This single-centre study therefore suggests that a single measure-
The "cut off" value for NT-proBNP was defined as the 85th centile of subjects with an LV ejection fraction \( \leq 40\% \) (357 \( \text{pmol/L} \)).

AUC = area under the curve in a receiver operating characteristic curve.

### Utility of NT-proBNP in the diagnosis of acute dyspnea in the urgent care setting

The utility of BNP in the diagnosis of patients with dyspnea presenting to an urgent care setting appears to be well-established.\(^{14,15}\) Evidence supporting the use of NT-proBNP in a similar setting is still evolving. The DAPIC study is a prospective study of 100 patients who presented to the emergency department in Hospital de Sant Pau, Barcelona, Spain. Final diagnosis was determined on the basis of department data sheets, echocardiography, and pulmonary function tests. NT-proBNP levels (Elecsys) were obtained on admission, at 24-hours, and on day 7. The final diagnosis was based on dyspnea with ventricular dysfunction and was, therefore, classified into dyspnea with normal ventricular function, "masked" HF, and decompensated HF. The initial diagnosis made by the emergency staff was incorrect in 18 patients (20.2%) and HF was missed in 17 patients.

Blood levels of NT-proBNP were higher in:
- "masked" HF (978±363 \( \text{pmol/L} \)),
- patients with normal ventricular function had lower levels (50±15 \( \text{pmol/L} \), pe.001 and pe.01, respectively.

The area under the ROC curve for NT-proBNP was 0.961 (95% CI, 0.818 - 0.996, pe.001). In multiple logistic regression analysis, a value of NT-proBNP >115 \( \text{pmol/L} \) was the strongest independent predictor of ventricular dysfunction (RR = 45.4, 95% CI, 4.5-452.3).

### NT-proBNP in outpatients with dyspnea of unknown origin: threshold levels for HF in the ambulatory primary healthcare sector

The clinical diagnosis of HF can be difficult, particularly in the primary care setting. Accordingly, the usefulness of plasma NT-proBNP for exclusion of HF was recently evaluated in 345 consecutive patients in Denmark who presented to their general practitioner because of dyspnea (Neilsen LS et al, unpublished data). These patients were referred to an outpatient dyspnea clinic where extensive testing was available, and further referral to tertiary care centres if required, in order to establish or rule out a diagnosis of HF.\(^{13}\) Without the influence of NT-proBNP, a final diagnosis of HF was made in the dyspnea clinic in 24% of the patients, whereas the general practitioners made a clinical diagnosis of HF in 54% of patients and missed the diagnosis in 20% of patients. In patients with a final diagnosis of HF, NT-proBNP (189±270 \( \text{pmol/L} \) or 1598±14202 \( \text{pg/mL} \)) was significantly higher than in patients with no HF (17±38 \( \text{pmol/L} \) or 144±321 \( \text{pg/mL} \), pe.001). Receiver operating characteristics curves were drawn to determine NT-proBNP threshold levels for HF in the 287 patients aged \( \geq 50 \) years. NT-proBNP values of <11 \( \text{pmol/L} \) (93 \( \text{pg/mL} \)) for males and <17 \( \text{pmol/L} \) (144 \( \text{pg/mL} \)) for females excluded HF in both males and females with a negative predictive value of 97%, while the sensitivity was 96% for males and 94% for females. The positive predictive value was 57% in males and 48% in females, with a specificity of approximately 70% for both sexes.

These preliminary data confirm that the diagnosis of HF in a primary care setting is difficult to exclude if based on clinical assessment alone and that NT-proBNP appears to be a promising marker for exclusion of HF in a population aged \( \geq 50 \) years with dyspnea. However, recommendations for the widespread use of NT-proBNP to exclude HF awaits prospective confirmation of the threshold levels identified.

#### Table 2: Performance characteristics of plasma NT-proBNP levels for the detection of left ventricular ejection \( \leq 40\% \) in unselected patients in a city hospital\(^{12}\)

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=2193)</th>
<th>Patients with symptoms or signs of HF (n=928)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>73%</td>
<td>78%</td>
</tr>
<tr>
<td>Specificity</td>
<td>82%</td>
<td>76%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>24%</td>
<td>30%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98%</td>
<td>96%</td>
</tr>
<tr>
<td>AUC</td>
<td>0.85</td>
<td>0.84</td>
</tr>
<tr>
<td>Prevalence of LV ejection fraction ( \leq 40% )</td>
<td>7%</td>
<td>12%</td>
</tr>
</tbody>
</table>
Table 3: Performance of BNP and NT-proBNP in the diagnosis of HF in patients with acute dyspnea\(^17\)

<table>
<thead>
<tr>
<th></th>
<th>Optimal value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>AUC</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP (Triage)</td>
<td>204 pg/ml</td>
<td>94%</td>
<td>70%</td>
<td>62%</td>
<td>96%</td>
<td>0.88</td>
<td>78</td>
</tr>
<tr>
<td>NT-proBNP (Elecsys)</td>
<td>340 pmol/L</td>
<td>82%</td>
<td>87%</td>
<td>76%</td>
<td>90%</td>
<td>0.89</td>
<td>85</td>
</tr>
</tbody>
</table>

AUC = area under the receiver operator characteristics curve

NT-proBNP as a prognostic marker in patients with HF

There are increasing data to suggest that NT-proBNP may be a useful prognostic marker in patients with HF. In a recent report of 91 patients with advanced HF started on double-blind ß-blockade therapy and followed to 24 months, both plasma BNP and NT-proBNP levels were independently related to mortality.\(^18\) Recently, investigators from the University of Glasgow prospectively studied 128 consecutive patients with advanced HF referred for consideration of cardiac transplantation.\(^19\) Patients were followed for a mean of 280 days. The median NT-proBNP was 1498 (544-3883) pmol/L. The only univariate and multivariate predictor of all-cause mortality was an NT-proBNP level above median value [relative risk (RR)=5.0 (95% CI, 1.7-22.4), p=0.002].

The value of NT-proBNP to predict adverse clinical events in patients with HF was prospectively evaluated in the European cohort of the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) study that enrolled patients with severe HF. Baseline plasma NT-proBNP values were obtained and ranged from undetectable to 9,525 pmol/L. By univariate and multivariate Cox regression analysis, NT-proBNP, by absolute values, classified by a median value of ±323 pmol/L, or by tertiles (<199, 199-504, and >504 pmol/L), was found to be a powerful predictor of all-cause mortality, regardless of treatment with carvedilol. Interestingly, subgroup analysis revealed that NT-proBNP levels were significantly higher in patients with ischemic than in those with non-ischemic etiology, and also higher in patients with elevated (≥125 pmol/L) serum creatinine (741 vs. 403 pmol/L, p=0.0001). Although most of the currently available data on the use of NT-proBNP as a prognostic maker in HF are still preliminary, these data, derived for the most part from prospective evaluations, appear very promising.

NT-proBNP in HF: therapy decision and monitoring

The possibility of using plasma NT-proBNP measurements to determine the appropriate timing for introducing and titrating therapy for HF is one of the most exciting potential clinical applications of plasma natriuretic peptide measurements. In an earlier study, Troughton et al randomized 69 patients with impaired systolic function and symptomatic HF to receive treatment guided by either plasma NT-proBNP level (NT-proBNP group, treatment goal <200 pmol/L) or standardized clinical assessment (clinical group).\(^20\) During follow-up (median 10 months), there were fewer total CV events in the NT-proBNP group than in the clinical group. At 6 months, 27% of patients in the NT-proBNP group and 53% in the clinical group had experienced a first CV event (p = 0.03). The authors concluded that NT-proBNP-guided treatment of HF reduced total CV events and delayed time to first event compared with clinically-guided treatment. It should be noted, however, that the treatment strategies employed at the time this study was conducted primarily included optimal doses of ACE inhibitors and loop diuretics, with the addition of digoxin, and vasodilators (eg, nitrates and calcium channel blockers). Importantly, ß-blockers were not administered.

The value of NT-proBNP in predicting the response to ß-blockade treatment with carvedilol was examined in the Australian-New Zealand Heart Failure Study.\(^21\) Among a broad array of neurohormonal measurements taken at the time, it was found that an increased plasma NT-proBNP level above the median was an independent predictor of an adverse clinical outcome and that patients benefited from therapy with carvedilol versus placebo (Figure 2). Conversely, in patients with NT-proBNP levels that were lower than median, carvedilol had no impact on clinical outcomes. A preliminary report from Glasgow and a recent presentation at the ACC meeting suggest that an NT-proBNP measurement taken before commencing ß-blockade therapy was an independent predictor of therapy tolerance (ie, the higher the level, the less likely the patient was able to tolerate the therapy).\(^22\) However, it is in this group – those with high NT-proBNP levels – that ß-blockade therapy may be of most benefit, suggesting that the therapy would require extremely slow and careful introduction.

The BATTLE-SCARRED trial is a study comparing NT-proBNP-guided versus clinically-guided therapy. In this trial, contemporary therapy including ß-blockers is being employed. The study is still ongoing, but some baseline data are available. The mean LV ejection fraction is 41.8%. Forty-three percent of...
patients have LV ejection fractions <40%, with mean NT-proBNP levels of 305±265 pmol/L, while 57% of patients have LV ejection fractions >40% and mean NT-proBNP levels of 257±210 pmol/L. Until further prospectively gathered data are available – and they are likely to be forthcoming – hormone-guided therapy using BNP or NT-proBNP measurements is not ready to be routinely employed.

**NT-proBNP in the acute coronary syndrome**

The potential utility of NT-proBNP as a prognostic marker in post-MI patients has recently been evaluated. In 121 MI patients followed for 24 months, plasma NT-proBNP levels obtained at 2- to 4-days post-MI (≥160 pmol/L) had sensitivity, specificity, positive, and negative predictive values of 91%, 72%, 39%, and 97%, respectively, for the prediction of death. These values were superior to other neurohormones measured, including catecholamines, C-terminal and N-terminal ANP, and LV ejection fraction.23 Moreover, by multivariate analysis, NT-proBNP provided predictive information for death and the development of HF independent of age, sex, LV ejection fraction, prior history of HF, and MI.

The potential utility of NT-proBNP has also been demonstrated in a broad spectrum of patients with acute coronary syndrome (ACS). In a recent study of 609 patients with ACS, including 204 with ST-elevation MI, 220 with non-ST elevation MI, and 185 with unstable angina,24 blood for NT-proBNP level was drawn at a median of 3 days post-admission. At a median of 51 months, 14% of the patients had died. Median NT-proBNP levels were significantly higher in patients who did not survive than in survivors (1306 versus 442 pmol/L, p < 0.001). In a multivariate analysis, NT-proBNP added prognostic information above and beyond Killip class, patient age, and LV function. Furthermore, adjustment for peak troponin T levels did not alter the relationship between NT-proBNP and mortality. Similar conclusions were drawn from a study in 755 patients with chest pain and without ST-segment elevation.25 Patients were followed for a median of 40 months. Median NT-proBNP was 400 (111 to 1646) pg/mL. Compared to the lowest quartile (113-400 pg/mL), patients in the second (113-400 pg/mL), third (401-1653 pg/mL), and fourth quartile (≥1654 pg/mL) had a relative risk of subsequent death of 4.2, 10.7, and 26.6, respectively (all significant). When incorporated into a Cox regression model including ECG, clinical background, and troponin T, elevated NT-proBNP was independently associated with a poor prognosis.

A recent, large-scale, multicentre study that involved 1756 patients with angina and electrocardiographic evidence of ischemia admitted to 31 coronary care units in Italy has yielded very similar findings.26 The relative risk of subsequent death at 30 days increased with quartiles of NT-proBNP levels drawn at a median of 3 hours after the onset of angina.

New, but yet to be published data from substudies of large multicentre trials in ACS, including FAST, GUSTO IV, and FRISC II, have contributed to the further understanding of NT-proBNP as an independent prognostic marker, as well as a potential marker for response to therapy. In GUSTO-IV, NT-proBNP was analyzed in 6808 patients.27 As with previous studies, the one-year mortality increased with increasing quartiles of NT-proBNP. In multiple logistic regression analysis of a large number of known clinical risk factors, NT-proBNP and troponin T were significant independent predictors of 30-day mortality, and combined mortality and reinfarction. Mortality was 0.7% with troponin T ≤0.1 µg/L and NT-proBNP <317 pg/mL (first tertile), and increased to 14.5% with troponin T >0.1 µg/L and NT-proBNP >1201 pg/mL (third tertile). In FRISC II, NT-proBNP and the pro-inflammatory cytokine interleukin-6 (IL-6) were measured.28 Mortality was reduced by an early invasive strategy only in patients with increased NT-proBNP and IL-6 levels.

Published and preliminary data thus far indicate that in patients with ACS, NT-proBNP measurement can provide useful prognostic information beyond conventional risk markers.

**Potential clinical use of NT-proBNP in other disease states**

In addition to HF and ACS, the use of NT-proBNP has also been explored in other clinical conditions. In 73 consecutive patients diagnosed with pulmonary embolism (PE), BNP and NT-proBNP were measured within 4 hours of admission.29,30 NT-proBNP was lower in the 53 patients with benign clinical outcomes (median 121, range 16-34,802 pg/mL) than in the 20 patients with adverse clinical outcomes including death or need for cardiopulmonary resuscitation, mechanical ventilation, use of pressors, thrombolysis, and surgical embolectomy (median 4,250, range 92-49,607 pg/mL, p<0.0001). The negative predictive value of NT-proBNP levels <500 pg/mL was 97% (95% CI, 84%-99%). The area under the ROC curve for the prediction of adverse outcome was 0.90 for NT-proBNP and 0.83 for BNP. NT-proBNP remained an independent predictor for adverse clinical outcome (RR 14.6, 95% CI,15-139; p<0.02) after adjusting for severity of PE, troponin T levels >0.01 µg/mL, age >70 years, gender, and history of HF.

Data on NT-proBNP and hypertension have only recently begun to emerge. In a recently presented substudy of the LIFE...
study,\textsuperscript{31} a modest correlation between NT-proBNP and LV mass determined by echocardiography was reported. However, changes over 1 year in NT-proBNP were not related to changes in echocardiographic variables. In another LIFE substudy,\textsuperscript{32} NT-proBNP was found to be a strong predictor of combined CV death, non-fatal stroke, and non-fatal MI in patients with hypertension and LV hypertrophy, especially in the subgroup without overt CV disease. Finally, another study in patients with valvular aortic stenosis demonstrated increasing NT-proBNP levels in parallel with increasing peak aortic gradient and decreasing aortic valve area.\textsuperscript{33}

\section*{Summary}

In summary, NT-proBNP is rapidly emerging as a useful marker in a variety of cardiovascular disorders, most notably HF and ACS. The use of NT-proBNP offers several theoretical advantages over BNP. Although much of the data regarding the clinical utility of NT-proBNP are still preliminary, they appear to be very promising. Further studies to better define the optimal cut-off points in different patient populations will likely improve the utility of NT-proBNP across a broad spectrum of patients with cardiovascular disorders.

\section*{References}


Dr. Moe discloses that he serves on the Medical Advisory Committee for Roche Diagnostics.

SNELL Medical Communication acknowledges that it has received an unrestricted educational grant from Roche Diagnostics Canada to support the distribution of this issue of Cardiology Scientific Update. Acceptance of this grant was conditional upon the sponsors’ acceptance of the policy established by the Division of Cardiology and SNELL Medical Communication guaranteeing the educational integrity of the publication. This policy ensures that the author and editor will at all times exercise unrestricted, rigorous, scientific independence free of interference from any other party.