

# Natriuretic Peptides (BNP and NT-proBNP) and Cardiovascular Disease Risk

Robert H. Christenson, Gary L. Myers, and Gerald R. Cooper

## RECOMMENDATIONS FOR BNP AND NTproBNP

Based on a thorough review of the published literature, the following are recommendations for the clinical use and measurement of BNP and NTproBNP in assessing risk for CHD and stroke in primary prevention.

### *Recommendation 1*

Increased B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) concentrations are associated with increased mortality in the next 2 to 7 years in community-based populations. However, the benefits of therapy based on these measurements are uncertain. Measurement for CVD risk assessment in the primary prevention setting is unwarranted.

**Classification of recommendation:** III (against measurement);

**Level of evidence:** B.

### *Recommendation 2*

More research should be performed to determine if BNP and NT-proBNP measurements are useful in identifying individuals who are at increased risk of developing heart failure and might benefit from therapies for prevention of heart failure and CVD.

**Classification of recommendation:** I

**Level of evidence:** C

### *Recommendation 3*

Manufacturers of reagents and kits for measurement of the BNP and NT-proBNP should be in compliance with current specifications developed by government and professional organizations, such as the IFCC.

**Classification of recommendation:** I

**Level of evidence:** C

### *Recommendation 4*

Laboratorians, clinicians, and manufacturers involved in utilizing and/or producing natriuretic peptide assays must work together to assure that all stakeholders are properly educated regarding preanalytical (eg, biological variation, specimen stability), analytical (the impact of various proBNP-derived peptides forms on assays, methodological variation of BNP results), and postanalytical (appropriate reference intervals, decision limits, and confounding clinical conditions) issues.

**Classification of recommendation:** I

**Level of evidence:** C

## BACKGROUND

The heart must be viewed as both a biological pump that cycles approximately 100,000 times per day and as an important hormone producing organ that produces biochemical signals that are antagonists for the sympathetic nervous system and the renin angiotensin aldosterone axis (1-3). Physiologically, there are a number of structurally and functionally related heart hormones manufactured by human cardiocytes. These include atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP) and their respective N-terminal (NT) metabolic peptides termed NT-proANP and NT-proBNP. Release of the natriuretic peptides is stimulated by hemodynamic stress; these hormones have powerful diuretic, natriuretic, and vascular smooth muscle relaxing actions (1-3). Because of their central pathophysiological role in the cardiovascular system, the natriuretic peptides are elevated in conditions characterized by wall stretch, ventricular dilation, and/or increased pressures resulting from excess fluid retention (2). Activation of the natriuretic system normally functions to reduce fluid blood volume and blood pressure.

BNP and its associated metabolite NT-proBNP have emerged as biomarkers of hemodynamic stress in acutely ill patients. Compared to ANP, BNP has a 2- to 3-fold more powerful natriuretic and blood pressure lowering effect on a molar basis (4). Under physiologic conditions, BNP concentrations are lower than ANP; however as the severity of hemodynamic stress

increases, plasma BNP levels increase more than corresponding ANP values (5). There is a positive correlation between blood BNP concentrations and left ventricular end diastolic pressure and inverse correlation to left ventricular function (6). In a study by Cowie et al (7), BNP showed the greatest predictive power as an indicator of heart failure when compared with either ANP or NT-proANP. Further, in the assessment of ventricular dysfunction and prediction of mortality in patients with severe heart failure, BNP was advocated as a better biomarker than either NT-proANP or ANP (8). BNP and NT-proBNP have emerged as the preferred biomarkers for assessing heart-related stress.

Regulation of BNP synthesis and secretion occurs mainly at the gene level; under physiologic conditions, BNP is preferentially produced and secreted in the ventricles of the heart without storage in granules (9,10). However, both ANP and BNP can be synthesized in either the atrium or ventricles, or both, under pathologic conditions; chronic fluid overload may cause rapid BNP production in both heart chambers, and production in the atrium may exceed the amount of ANP (11,12). According to the conventional model, human BNP is synthesized within the myocytes from the 134-aa precursor preproBNP. Upon stimulation for release, a 26-aa signal peptide sequence is cleaved from the N-terminus of preproBNP to form proBNP<sub>1-108</sub>. During release into circulation, the proBNP<sub>1-108</sub> prohormone is further cleaved by corin, a membrane-bound serine protease, into an N-terminal pro-BNP<sub>1-76</sub> fragment termed NT-proBNP and the active 32-peptide, C-terminal pro-BNP<sub>77-108</sub> hormone-termed BNP. Although this model of BNP and NT-proBNP release is widely accepted, there is a body of evidence that the forms of BNP and NT-proBNP released into circulation are not as well understood as previously thought. A recent study utilizing innovative liquid chromatographic and mass spectrometry technology conclusively demonstrated that cross-reactive species contribute substantially to BNP measurements in patients with severe heart failure (13). It is important to note, however, that this finding does not mitigate clinical trials showing the diagnostic and prognostic utility of BNP and NT-proBNP measurements by immunoassay (13).

Table 9 shows the performance of BNP and NT-proBNP in the acute setting for use in diagnosing decompensated heart failure (14). This good performance and the basic physiology of the biomarkers also prompted interest in utilizing these biomarkers as tools for detecting left ventricular systolic dysfunction (Table 9) (14). Although BNP is more thoroughly studied, there is no evidence that for clinical purposes the accuracy of

BNP differs from that of NT-proBNP for the cross-sectional applications of decompensated heart failure diagnosis or assessment of left ventricular systolic dysfunction (14).

Concerns regarding the equivalency of BNP and NT-proBNP in the setting of renal insufficiency have been raised due to the notion that NT-proBNP clearance is more dependant on renal function than BNP (15). This is of significant concern since 33% to 57% of patients with heart failure have impairment of renal function (16-18). Recent data have shown that renal extraction of NT-proBNP and BNP is comparable across a broad range of renal function (19,20). Also, direct comparison of NT-proBNP and BNP in a large observational cohort of patients showed that the biomarker assays examined appeared to be equivalent tools for diagnosis of decompensated heart failure in patients with renal insufficiency as well as in patients with GFR > 60 mL/min (21).

Patients having decompensated heart failure diagnosis and/or left ventricular systolic dysfunction are clearly at high risk for adverse short- and long-term outcomes. Also, these patients are likely to have ischemic heart disease as this is the most common cause of heart failure. For this reason, the committee investigated evidence to examine if BNP and NT-proBNP measurements can add information to traditional risk factors for primary prevention assessment.

## DISCUSSION OF EVIDENCE

The committee identified several longitudinal studies (22-25) which adjusted for appropriate risk factors such as age, total cholesterol, smoking habit. Table 10 presents a summary of studies which led to the recommendations for BNP and NTproBNP. Evidence indicates that measurement of BNP and NTproBNP provide prognostic information of mortality and first cardiovascular events beyond traditional risk factors (22,23). Also, NT-proBNP was a stronger risk biomarker in nonhospitalized individuals 50 to 89 years old for predicting CVD and death than CRP (22). Excess risk was apparent at levels well below current thresholds used to diagnose decompensated heart failure (22, 23). Also, the association of BNP and NT-proBNP with increased risk of cardiac morbidity and mortality also held for very elderly patients (24). As in the cross-sectional studies included in a National Health Service technology assessment report (14), there are indications that NT-proBNP performs equivalently with BNP for detecting left

**Table 9. BNP and NT-proBNP Performance for Diagnosis of DHF and Detecting LVSD**

	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)
DHF Diagnosis			
BNP	0.91 (0.90-0.93)	0.73 (0.71-0.75)	35.66 (17.11-74.30)
NT-proBNP	0.91 (0.88-0.93)	0.76 (0.75-0.77)	39.86 (18.13-87.64)
LVSD Detection			
BNP	0.88 (0.84-0.91)	0.62 (0.60-0.63)	10.74 (6.51-17.72)
NT-proBNP	0.84 (0.80-0.88)	0.65 (0.64-0.67)	14.96 (10.69-20.94)
Abbreviations: BNP, NT-proBNP, DHF, decompensated heart failure; LVSD, left ventricular systolic dysfunction; DOR, diagnostic odds ratio. NOTE. Data from NHS Health Technology Assessment (14).			

**Table 10. BNP and NT-proBNP Studies With Long-Term Outcome**

Reference	Study Design	Follow up	Endpoints	Adjusted hazard ratio or OR (95% CI)	Assay Used	Comparison Biomarker(s)
22 (Wang et al)*	Community based cohort	Mean: 5.2 years	Death Firstst major CV event Heart failure Htrial fibrillation Stroke or TIA CHD events	1.62 (1.08-2.42) 1.76(1.06-2.92) 3.07 (1.51-6.26) 1.91 (1.13-3.25) 1.99(1.09-3.62) 1.30 (0.79-2.15)	Shiono RIA BNP assay	NT-ANP
24 (Ueda et al) **	Cohort of elderly patients (mean 85 years)	2 years	Mortality Cardiac hospitalization	Each 50 pg/mL increased rate of mortality 1.4-fold (1.2-1.6) Each 50 pg/mL increased rate of cardiac events 1.6-fold (1.2-2.1)	Shiono RIA BNP assay	None
23 (Kistorp et al)***	Population based cohort	Mean: 5-years	All cause mortality First major cardiac event without CVD at baseline	HR 1.96 (1.21-3.19) HR 3.24 (1.80-5.79) (Top quartile of NT-proBNP results)	NT- proBNP Roche	hsCRP, 1.17 (0.95-1.43), 1.46 (0.89-2.24); Alb/creat, 1.38 (1.16-1.65), 1.88 (1.18-2.98); hsCRP, 1.15 (0.88-1.51), 1.02 (0.56-1.85); Alb/creat, 1.57 1.26-1.95), 2.32 ((1.33-4.05)
25 (McKie et al)****	Community based cohort	5.6 years	All cause mortality	Adjusted: NT-proBNP 1.63 (1.25-2.13);	BNP Biosite and Biosite: 1.50 (1.15-1.95); 1.39 ) (1.10-1.74)	Two BNP assays and 1 NT-proBNP assay Shionogi, NT- proBNP Roche

\*Age, sex, presence or absence of hypertension, ratio of total to HDL cholesterol, smoking status, presence or absence of diabetes mellitus, BMI, serum creatinine.

\*\*Age, sex, BMI, Blood pressure, heart rate, total protein, creatinine, Hb A1c, total cholesterol, ischemic heart disease, EKG abnormality, history of stroke, ADL score

\*\*\*Age, sex, current smoking, diabetes mellitus, hypertension, and ischemic heart disease, total cholesterol, and serum creatinine.

\*\*\*\*Age and sex adjusted; Model 1: age, sex, total cholesterol, and serum creatinine, presence of diabetes mellitus, hypertension, and coronary artery disease.

Abbreviations: BNP, brain (B-type) natriuretic peptide; NT proBNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; TIA, transient ischemic attack; NT-ANP, N-terminal atrial natriuretic peptide; CV, cardiovascular; CHD, coronary heart disease; hsCRP, Alb, albumin; creat, creatinine.

ventricular dysfunction (25). There are data suggesting that NT-proBNP and BNP may be potentially useful for predicting future events and that these measurements may reveal underlying cardiac remodeling secondary to diverse CVD entities (25). However, there is currently no evidence that treatment or intervention based on the increased risk implied by these biomarkers improves patient outcomes. Thus, the committee advises against routine measurement in the primary prevention population. The potential benefit of therapies may be substantial and should be considered an important area of future research.

Regarding possible cutpoints to utilize for identifying high risk patients, it must be noted that reference intervals for BNP and particularly NT-proBNP show a dependence on age and sex. Of interest, a nested case-control study was performed in a large cohort of men (> 10,000), age 35 to 59 years, with a median follow-up of 2.66 years. A highly significant difference in NT-proBNP values ( $P < .0001$ ) was found between cases having coronary events (median, 48.5 pg/mL; interquartile range, 26.4 to 116.6 pg/mL) and controls with no events (median, 30.0 pg/mL; 1 interquartile range, 9.5 to 47.6 pg/mL) (26). Thus, the major finding of this study was that NT-pro-BNP is a strong pre-

dictor of coronary events in working men after adjustment for conventional risk factors (26). Another study of community based older individuals between 50 to 89 years found that a NT-proBNP value exceeding the 80th percentile value of 655 ng/L corresponding to an adjusted HR for mortality of 1.96 (95% CI, 1.21 to 3.19) (23). Based on this information, the committee suggests that the 80th percentile of the control reference population may be useful as a cutpoint, but requires further validation.

As with any biomarker, analytical specifications for BNP and NT-proBNP must be driven by a balance between physiology and clinical utilization. A recent report aimed at improving the quality of immunochemical measurements of BNP and NT-proBNP was recently published by the IFCC Committee on Standardization of Markers of Cardiac Damage (26). The recommendations proposed were intended for use by manufacturers of commercial assays, by clinical laboratories using those assays, by clinical trial groups and research investigators, as well as by regulatory agencies such as the United States Food and Drug Administration (27). This document was developed by experts who reviewed and abstracted the scientific literature pertaining to the needed quality specifications for BNP and NT-proBNP assays. These evidence-based recommendations encourage manufacturers of BNP and NT-proBNP diagnostics to include information in their package inserts that includes assay design, preanalytical performance characteristics, analytical performance characteristics, and clinical performance. In addition, regulatory agencies are encouraged to adopt a minimal and uniform set of criteria to help guide manufacturers seeking clearance for new and/or improved assays.

Knowledge in the context of the natriuretic is evolving rapidly. Utilization of BNP and NT-proBNP measurements is complicated by preanalytical issues. Stability at room temperature facilitates handling of specimens in routine laboratories in the preanalytical phase, and NT-proBNP is more stable in vitro than BNP (28). Serum or plasma NT-proBNP measurements are stable for 7 days at room temperature, 10 days at 4° C, and at least several months at -20° C or lower temperatures (29,30). Five freeze-thaw cycles do not diminish NT-proBNP concentrations significantly (29,31). BNP stability is dependent on the specific assay (28,32). At room temperature BNP measurements appear to diminish soon after collection, at 4° C levels are stable for about 4 hours, and at -20° C or colder BNP levels appear to decrease significantly within a few weeks (28). Other preanalytical issues, such as, biological variability, age, and sex differences, analytical issues such as assay performance, as well as post analytical issues such as result reporting and recognizing that different diseases and patient populations must utilize the test differently. Communication, cooperation, and education, both initially and continuous learning, are vital components for appropriate utility of BNP and NT-proBNP measurements in patient care.

## REFERENCES

1. Clerico A, Iervasi G, Mariani G. Clinical relevance of the measurement of cardiac natriuretic peptide hormones in humans. *Horm Metab Res* 1999; 31:487-498.
2. Boomama F, Van der Meiracker AH. Plasma A- and B-type natriuretic peptides: physiology, methodology and clinical use. *Cardiovasc Res* 2001; 51:442-449.
3. Azzazy HM, Christenson RH. B-type natriuretic peptide: physiologic role and assay characteristics. *Heart Fail Rev* 2003; 8:315-20.
4. Janssen WM, de Zeeuw D, van der Hem GK, de Jong PE. Antihypertensive effect of a 5-day infusion of atrial natriuretic factor in humans. *Hypertension* 1989; 13:640-646.
5. Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans: Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* 1991; 87:1402-1412.
6. Maeda K, Tsutamoto T, Wada A, Hisanaga T, Kinoshita M. Plasma Brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. *Am Heart J* 1998; 135:825-832.
7. Cowie MR, Struthers AD, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, Sutton GC. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. *Lancet* 1997; 350:1349-1353.
8. Sagnella GA. Measurement and significance of circulating natriuretic peptides in cardiovascular disease. *Clin Sci* 1998; 95:519-529.
9. De Bold AJ, Bruneau BG, Kuroski de Bold ML. Mechanical and neuroendocrine regulation of the endocrine heart. *Cardiovasc Res* 1996; 31:7-18.
10. Mair J, Hammerer-Lercher A, Puchendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. *Clin Chem Lab Med* 2001; 39: 571-588.
11. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, Ogawa H, Okumura K, Mukoyama M, Nakao K. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 1994; 90:195-203.
12. Luchner A, Stevens TL, Borgeson DD, Redfield M, Wei CM, Porter JG, Burnett JC Jr. Differential atrial and ventricular expression of myocardial BNP during evolution of heart failure. *Am J Physiol* 1998; 274:H1684-H1689.
13. Hawkrige AM, Heublein DM, Bergen HR 3rd, Cataliotti A, Burnett JC Jr, Muddiman DC. Quantitative mass spectral evidence for the absence of circulating brain natriuretic peptide (BNP-32) in severe human heart failure. *Proc Natl Acad Sci U S A* 2005; 102:17442-7.
14. Craig J, Bradbury I, Cummins E, Downie S, Foster L, Stout A. The use of B-type natriuretic peptides (BNP and NT-proBNP) in the investigation of patients with heart failure. *Health Technology Assessment Report 6. NHS Quality Improvement Scotland*, 2005.
15. McCullough PA, Sandberg KR. Sorting out the evidence on natriuretic peptides. *Rev Cardiovasc Med* 2003; 4(suppl 4):S13-S19.
16. Mahon NG, Blackstone EH, Francis GS, Starling RC, 3rd, Young JB, Lauer MS. The prognostic value of estimated creatinine clearance alongside functional capacity in ambulatory patients with chronic congestive heart failure. *J Am Coll Cardiol* 2002; 40:1106-13.
17. McAlister FA, Ezekowitz J, Tonelli M, Armstrong PW. Renal Insufficiency and Heart Failure: Prognostic and Therapeutic Implications From a Prospective Cohort Study. *Circulation* 2004; 109:1004-9.

18. Hillege HL, Nitsch D, Pfeffer MA, et al. Renal function as a predictor of outcome in a broad spectrum of patients with heart failure. *Circulation* 2006; 113:671-8.
19. Kimmenade RR, Bakker JA, Houben AJ, et al. Renal handling of BNP and NT-proBNP in hypertensive subjects. *Circulation*. 2005; 112:II-601 (abstr).
20. Schou M, Dalsgaard MK, Clemmesen O, Dawson EA, Yoshiga CC, Nielsen HB, Gustafsson F, Hildebrandt PR, Secher NH. Kidneys extract BNP and NT-proBNP in healthy young men. *J Appl Physiol*. 2005; 99:1676-80.
21. deFilippi CR, Seliger S, Maynard S, Christenson RH. Impact of renal disease on natriuretic peptide testing for diagnosing decompensated heart failure and predicting mortality. *Clin Chem* 2007; 53:1511-9.
22. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. *JAMA* 2005; 293:1609-1616.
23. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004; 350:655-663.
24. Ueda R, Yokouchi M, Suzuki T, Otomo E, Katagiri T. Prognostic value of high plasma brain natriuretic peptide concentrations in very elderly persons. *Am J Med* 2003; 114:266-270.
25. McKie PM, Rodeheffer RJ, Cataliotti A, Martin FL, Urban LH, Mahoney DW, Jacobsen SJ, Redfield MM, Burnett JC Jr. Amino-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide: biomarkers for mortality in a large community-based cohort free of heart failure. *Hypertension* 2006; 47:874-80.
26. de Sutter J, de Bacquer D, Cuypers S, Delanghe J, de Buyzere M, et al. Plasma N-Terminal Pro-Brain Natriuretic Peptide Concentration Predicts Coronary Events in Men at Work: a Report from the BELSTRESS Study. *European Heart Journal*, 2005. 26:2644-2649.
27. Apple FS, Panteghini M, Ravkilde J, Mair J, Wu AH, Tate J, Pagani F, Christenson RH, Jaffe AS. Quality specifications for B-type natriuretic peptide assays. *Clin Chem* 2005; 51:486-93.
28. Ordonez-Llanos J, Collinson PO, Christenson RH. Amino-terminal pro-B-type natriuretic peptide: analytic considerations. *Am J Cardiol*. 2008; 101:9-15.
29. Sokoll LJ, Baum H, Collinson PO, Gurr E, Haass M, Luthe H, Morton JJ, Nowatzke W, Zingler C. Multicenter analytical performance evaluation of the Elecsys proBNP assay. *Clin Chem Lab Med* 2004; 42:965-72.
30. Yeo KT, Wu AH, Apple FS, Kroll MH, Christenson RH, Lewandrowski KB, Sedor FA, Butch AW. Multicenter evaluation of the Roche NT-proBNP assay and comparison to the Biosite Triage BNP assay. *Clin Chim Acta* 2003; 338: 107-15.
31. Barnes SC, Collinson PO, Galasko G, Lahiri A, Senior R. Evaluation of N-terminal pro-B type natriuretic peptide analysis on the Elecsys 1010 and 2010 analysers. *Ann Clin Biochem* 2004; 41:459-63
32. Christenson RH, Azzazy HM, Duh SH. Stability of B-type natriuretic peptide (BNP) in whole blood and plasma stored under different conditions when measured with the Biosite Triage or Beckman-Coulter Access systems. *Clin Chim Acta* 2007; 384:176-8.