Journal of the American College of Cardiology
© 2011 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 57, No. 12, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.09.075

EDITORIAL COMMENT

Natriuretic Peptide Assays Revisited

Do We Need Pro–B-Type Natriuretic Peptide?*

Michele Emdin, MD, PHD,†
Claudio Passino, MD,†‡ Aldo Clerico, MD†‡
Pisa, Italy

Discovery of the endocrine function of the heart (1), which exerts a fundamental role in regulating cardiovascular homeostasis through atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), has opened up a new field of research that has resulted in multiple clinical applications (2). The human *BNP* gene encodes for a pre-proBNP molecule of 134 amino acid residues, including a signal peptide of 26 amino acids. The BNP is cleaved out of a prohormone molecule of 108 amino acids, the $proBNP_{1\text{-}108}$ (proBNP). Before secretion from cardiomyocytes into blood, proBNP is split by some proteolytic enzymes (probably corin and/or furin) into 2 peptides: the biologically

See page 1386

inactive NH2-terminal peptide fragment $proBNP_{1–76}$ (NT-proBNP), and the COOH-terminal peptide fragment $proBNP_{77–108}$ (BNP), which is the active hormone, binding specific receptors (NPR-A, -B, -C) (2,3). In addition to bioactive BNP, a huge number of circulating proBNP-derived fragments can be identified by chromatographic procedures in human plasma (4), including the intact precursor $proBNP_{1–108}$, found in a significant amount in stage C and stage D heart failure (HF) patients (3–5). Liang et al. (6) demonstrated that proBNP, constituting a substantial portion of immunoreactive BNP in plasma of HF patients, possesses significantly lower biological activity than the 32-amino acid hormone.

The widespread diffusion of automated assays of BNP and NT-proBNP has furnished a novel tool for diagnosis, prognostic evaluation, and guide to treatment of overt HF, as well for the screening of asymptomatic left ventricular dysfunction in the general population (2,7), with a potential use in primary care (8). Recent evidence has challenged, however, the specificity of all commercial assays for BNP and NT-proBNP, indicating a significant cross-reactivity with the precursor $proBNP_{1–108}$, variable for each assay (9). This means that, beyond their proven diagnostic accuracy and prognostic value in HF, those assays are not specific for their declared target, with evident implications for the pathophysiological and clinical interpretation of data, not yet completely understood.

In this issue of the *Journal*, Macheret et al. (10) measured in the general community the circulating intact precursor $proBNP_{1–108}$ by means of a highly specific $proBNP_{1–108}$ sandwich immunoassay (11). They evaluated its ability to detect left ventricular systolic dysfunction, in comparison with commercial BNP and NT-proBNP assays, in a cohort of 1,939 subjects (672 healthy subjects and 1,267 stages A to C HF patients, ≥45 years of age), undergoing clinical and echocardiographic characterization. The method used is based on a specific monoclonal antibody recognizing the cleavage site of $proBNP_{1–108}$, without any significant cross-reactivity with either recombinant $NT\text{-}proBNP_{1–76}$ or synthetic $BNP_{1–32}$, combined with a polyclonal antibody directed against $BNP_{1–32}$ (11).

Macheret et al. (10) demonstrated that $proBNP_{1–108}$ was detectable in all subjects studied, and its level was dependent on sex, age, heart rate, and body mass index. Furthermore, they found that the degree of sensitivity and specificity of the proBNP assay for the detection of left ventricular dysfunction was comparable to 2 commercial assays for BNP and NT-proBNP (10).

This study confirms and extends previous evidence (5) that the intact precursor of biologically active BNP (i.e., the $proBNP_{1–108}$) circulates in plasma of both healthy subjects and HF patients, and underscores the diagnostic usefulness of its assay (5), which has been previously shown to hold prognostic value in patients with systolic HF, too (12). Moreover, circulating levels of $proBNP_{1–108}$ are age- and sex-dependent and progressively increase with disease severity in HF patients, as previously reported for BNP and NT-proBNP measured by commercial immunoassays (2,3,13).

Two potential drawbacks are evidenced by the Macheret study (10): the automated proBNP immunoassay is not commercially available, and may not be considered, at this moment, for current clinical use, especially in emergency departments. Moreover, this study was not able to demonstrate a better diagnostic accuracy of proBNP for left ventricular dysfunction, as compared with a currently used NT-proBNP assay.

However, the high specificity of this proBNP assay for targeted molecules and its overall good diagnostic accuracy as shown in this study pave the way for a future use of this biomarker, as an alternative to other BNP assays currently used for screening of left ventricular dysfunction in asymp-

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the †Fondazione G. Monasterio CNR-Regione Toscana, Pisa, Italy; and ‡Scuola Superiore Sant'Anna, Pisa, Italy. All authors have reported that they have no relationships to disclose.

tomatic subjects at intermediate to high risk for HF, or for differential diagnosis in patients with dyspnea. Evidence is growing, supporting an out-of-hospital use of natriuretic peptide assays: their implementation in screening programs and diagnostic algorithms in primary care might contribute to decrease the apparent disparity between the general practitioner and the specialist approach to disease management (8).

Indeed, the findings by Macheret et al. (10) indicate that the greater part of B-type natriuretic peptides assayed in HF patients is devoid of biological activity. As a whole, these results (10) support the hypothesis that the post-translational maturation processing of ventricular proBNP is not efficient in HF (2,15,16).

The *BNP* gene is expressed in both atrial and ventricular myocytes of normal and diseased hearts (1,2,14). Ventricular myocytes do not usually display any evident secretory granules at electron microscopy in normal heart, whereas some granules, similar to the atrial ones, can be found in samples of ventricular myocardium collected during surgery or in endocardial biopsies (2,3). That suggests that normal ventricular myocardium may produce only a limited amount of biologically active $BNP_{1–32}$ in response to an acute, efficacious stimulation, probably through a constitutive secretory pathway, while the amount of hormone produced and secreted after chronic stimulation in patients with HF might be greatly increased through an up-regulated secretory pathway (10,14). According to this hypothesis, a huge amount of immunoreactive BNP circulating in plasma of HF patients and measurable with conventional BNP and NT-proBNP immunoassays is not biologically active. That may add to the understanding of the resistance to the biological effects of natriuretic hormones (the "natriuretic paradox"), which plays an important role in the pathophysiology of sodium retention and systemic and renal vasoconstriction observed in severe HF, substantially contributing to disease progression (2,3,15).

At present, the exact reason for $proBNP_{1–108}$ processing deficiency in HF is unknown, but an enzymatic deficiency, either due to saturation or constitutive dysfunction, might underlie the progression from asymptomatic cardiac dysfunction to overt HF (2). The hypothesis of a "cardiac endocrine dysfunction" may support the high variability of clinical HF phenotypes, given a similar etiology and extent of cardiac damage/dysfunction. However, it is well known that infusion of the same ANP or BNP dose has a reduced natriuretic effect in HF patients, as compared to healthy subjects. This observation suggests that there are also other receptorial and post-receptorial mechanisms causing the resistance to natriuretic hormone action, including down-regulation of A and B natriuretic receptors, up-regulation of clearance NPR-C receptors, or post-receptor issues, such as increased activity of cyclic guanosine monophosphate-phosphodiesterase, which metabolizes cyclic guanosine monophosphate (2,16).

Conversely, it is theoretically conceivable that the COOH-terminal fragment including the cysteine bond (i.e., the active hormone $BNP_{1–32}$) may be produced even in vivo from the circulating intact precursor proBNP by plasma enzyme degradation. Although there is no demonstration that circulating proBNP can be processed in vivo in human blood, a soluble form of corin, a transmembrane serine protease able to cleave both proANP and proBNP, may be also capable of processing the circulating intact precursor of natriuretic hormones (17). Dong et al. (18) recently confirmed that soluble corin is measurable in human blood. Furthermore, these investigators reported that plasma corin levels are significantly lower in HF patients than in healthy controls and that the reduction in plasma enzyme correlates with the severity of the disease (18). These preliminary observations, if confirmed, suggest a more complex scenario regarding the "natriuretic paradox": the circulating intact precursor proBNP might be actually considered a prohormone, processed into the active peptide, when necessary (17,18). This hypothesis assumes that the peripheral processing of circulating proBNP would be submitted to regulatory rules, possibly altered with HF progression. This abnormality might be considered as a possible future therapeutic target, for example, by studying drugs inducing the cleavage of the prohormone into active BNP.

Although the assay of proBNP (a more stable molecule with higher molecular weight than derived peptides) has some theoretical advantages, from an analytical point of view, once automated assays will be available, 1 important issue could facilitate its diffusion in the clinical setting. As a future perspective, the simultaneous measurement in the same plasma sample with 2 methods, 1 specific for the intact precursor $proBNP_{1–108}$, and the other for active peptide $BNP_{1–32}$, could allow a more accurate estimate of the true activity of cardiac natriuretic hormone system as compared with the single assay of either peptide (4,9), likely extending our present understanding of pathophysiological and clinical changes due to cardiac endocrine dysfunction on HF evolution. Indeed, a recent study of ambulatory patients with chronic systolic HF showed that the combined assessment of conventional BNP and proBNP immunoassays provides additional information in determining the risk of adverse clinical outcomes, particularly in patients with low BNP values (12). Conversely, the proposal by Macheret et al. (10) of considering proBNP/NT-proBNP ratio, based on an electrochemiluminescent immunoassay of NT-proBNP, as a useful interpretative tool is questionable. Actually, this ratio does not reflect either the ratio between the prohormone and the biologically active hormone or the true relationship between the prohormone and NT-proBNP peptide level, because of the cross-reactivity of the NT-proBNP method used with proBNP, as mentioned by the authors themselves.

In summary, a $proBNP_{1–108}$ assay shows several features of the ideal biomarker (in vivo and in vitro stability, adequate analytical-functional sensitivity, reproducibility,

and accuracy) (11), whereas others are still lacking (complete assay automation, demonstration of low biological variation, reference range and cut-off values tested for sex, age, and ethnicity dependence, extensively proven good diagnostic and prognostic accuracy, evaluation of cost effectiveness). Our interest in this "novel" natriuretic peptide assay is in its specificity for the molecule targeted, whereas at present all conventional BNP and NT-proBNP immunoassays, showing some cross-reactivity with the intact precursor proBNP, may be considered, at the same time, accurate markers of HF presence and severity, and inaccurate indexes of BNP biological activity (9). Diagnostic manufacturers should set up specific immunoassay methods for biologically active $BNP_{1–32}$ peptide, to overcome this important drawback. Thereafter, designed studies would be necessary to estimate and compare the diagnostic and prognostic accuracy of specific assays for different B-type related peptides: BNP, NT-proBNP, and intact proBNP, used either alone or in combination.

Reprint requests and correspondence: Dr. Michele Emdin, Cardiovascular Medicine Division, Fondazione G. Monasterio CNR-Regione Toscana, Via Giuseppe Moruzzi 1, Pisa 56124, Italy. E-mail: emdin@ftgm.it.

REFERENCES

1. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 1981;28:89–94.
2. Clerico A, Recchia FA, Passino C, Emdin M. Cardiac endocrine function is an essential component of the homeostatic regulation network: physiological and clinical implications. Am J Physiol Heart Circ Physiol 2006;290:H17–29.
3. Goetze JP. Biosynthesis of cardiac natriuretic peptides. Results Probl Cell Differ 2010;50:97–120.
4. Seferian KR, Tamm NN, Semenov AG, et al. The brain natriuretic peptide (BNP) precursor is the major immunoreactive form of BNP in patients with heart failure. Clin Chem 2007;53:866–73.
5. Mogelvang R, Goetze JP, Schnohr P, et al. Discriminating between cardiac and pulmonary dysfunction in the general population with dyspnea by plasma pro-B-type natriuretic peptide. J Am Coll Cardiol 2007;23;50:1694–701.
6. Liang F, O'Rear J, Schellenberger U, et al. Evidence for functional heterogeneity of circulating B-type natriuretic peptide. J Am Coll Cardiol 2007;49:1071–8.
7. Hunt SA, Abraham WT, Chin MH, et al. 2009 focused update incorporated into the ACC/AHA 2005 guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2009;53:e1–90.
8. Emdin M, Fontana M, Poletti R, et al. Natriuretic peptide testing in primary care patients. Clin Chem Lab Med 2008;46:1533–42.
9. Luckenbill KN, Christenson RH, Jaffe AS, et al. Cross-reactivity of BNP, NT-proBNP, and proBNP in commercial BNP and NT-proBNP assays: preliminary observations from the IFCC Committee for Standardization of Markers of Cardiac Damage. Clin Chem 2008;54:619–21.
10. Macheret F, Boerrigter G, McKie P, et al. Pro–B-type natriuretic $peptide_{1–108}$ circulates in the general community: plasma determinants and detection of left ventricular systolic dysfunction. J Am Coll Cardiol 2011;57:1386–95.
11. Giuliani I, Rieunier F, Larue C, et al. Assay for measurement of intact B-type natriuretic peptide prohormone in blood. Clin Chem 2006;52:1054–61.
12. Dries DL, Ky B, Wu AH, Rame JE, Putt ME, Cappola TP. Simultaneous assessment of unprocessed ProBNP1-108 in addition to processed BNP32 improves identification of high-risk ambulatory patients with heart failure. Circ Heart Fail 2010;3:220–7.
13. Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of the cardiac natriuretic peptides: a review. Clin Chem 2004;50:33–50.
14. McGrath MF, de Bold AJ. Determinants of natriuretic peptide gene expression. Peptides 2005;26:933–43.
15. Chen HH. Heart failure: a state of brain natriuretic peptide deficiency or resistance or both! J Am Coll Cardiol 2007;49:1089–91.
16. Forfia PR, Lee M, Tunin RS, Mahmud M, Champion HC, Kass DA. Acute phosphodiesterase 5 inhibition mimics hemodynamic effects of B-type natriuretic peptide and potentiates B-type natriuretic peptide effects in failing but not normal canine heart. J Am Coll Cardiol 2007;49:1079–88.
17. Knappe S, Wu F, Masikat MR, et al. Functional analysis of the transmembrane domain and activation cleavage of human corin: design and characterization of a soluble corin. J Biol Chem 2003;278:52363–70.
18. Dong N, Chen S, Yang J, et al. Plasma soluble corin in patients with heart failure. Circ Heart Fail 2010;3:207–11.

Key Words: biomarker ■ BNP ■ heart failure ■ NT-proBNP ■ proBNP.