NATRIURETIC PEPTIDES AS MARKERS OF CARDIOVASCULAR DISEASE. THEIR USE IN DIAGNOSIS, PROGNOSIS AND THERAPY.

Adolfo J. de Bold, Ph.D.

Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of Ottawa and Cardiovascular Endocrinology Laboratory, University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4W7

The cardiac natriuretic peptides (NP) atrial natriuretic factor (ANF) (7,8) and brain natriuretic peptide (BNP)(17) are polypeptide hormones synthesized, stored and secreted by cardiac muscle cells (cardiocytes). These hormones are released from storage sites found in the atria of all mammals.

The biological properties of NP include the modulation of intrinsic renal mechanisms, the sympathetic nervous system, the renin-angiotensin-aldosterone system and other determinants of cardiovascular homeostasis. In addition, NP have potent growth regulatory actions on vascular smooth muscle.(7) Owing to these properties, the NP limit increases in extracellular fluid volume and blood pressure. The biological actions of NP are predominantly mediated through increases in cGMP in target cells via guanylyl cyclase-coupled receptors. Both ANF and BNP are agonists of the type A NP receptor. Intracellular cGMP receptors include cGMP-dependant protein kinases, cGMP-gated ion channels and cGMP-regulated cyclic nucleotide phosphodiesterases.(2,16) Steady-state NP circulating levels are maintained by a balance between their rate of production, their clearance by a clearance receptor, and neutral endopeptidase activity, which is particularly abundant in the brush border of the proximal convoluted tubules of the kidney.

Both ANF and BNP are synthesized by cardiocytes as preprohormones that are enzymatically processed to yield prohormones and, ultimately, hormones that are released into the circulation. In humans (Fig. 1), the prohormones proANF is a polypeptide that contains 126 amino acids (ANF$_{1-126}$) that is processed to ANF$_{1-98}$ (N-terminal ANF = NT-ANF) and ANF$_{99-126}$ (C-terminal ANF = CT-ANF). The latter is the biologically active portion. Human proBNP, on the other hand, is 108 amino acids long and it is processed to BNP$_{1-76}$ (N-terminal BNP = NT-BNP) and BNP$_{77-108}$ (C-terminal BNP = CT-BNP). Similarly to CT-ANF, CT-BNP is the biologically active peptide. These latter fragments are rapidly cleared from the circulation while their N-terminal portions have comparatively longer half-lives. From the clinical point of view therefore, the N-terminal fragments may best integrate events over time.

In pathophysiological states of the ventricular muscle such as hypertrophy, failure and inflammation, ventricular cardiocytes re-express their fetal capacity to produce ANF and BNP. However, even in pathophysiological states the main sources of NP are the atria and not the ventricles as claimed by BNP by some workers. Indeed, experimental data shows that the content of ANF and BNP as well as that of their respective mRNAs in atrial tissue is several orders of magnitude larger in atria than it is in the ventricles in both the normal and hhypertrophic ventricles even when

taking into account the much larger mass of the ventricles (28). In addition, cardiac catetheterization studies in humans (24) show that atrial-derived peptide contributes significantly to the elevation of plasma BNP level, reflecting atrial pressure and volume loading in left ventricular hypertrophy.

Much work has accumulated in recent years establishing the usefulness of determining plasma levels of ANF and BNP to help diagnose several cardiac pathologies. Results of this work clearly demonstrate the desirability of implementing the measurement of these hormones in the clinical setting from the diagnostic, prognostic, therapeutic efficacy and economic points of view.

Natriuretic Peptides and Cardiovascular Disease

Soon after the development of specific and sensitive radioimmunoassays (RIAs) for ANF in plasma, it was apparent that the circulating levels of this hormone were significantly elevated in a variety of clinical conditions, all of which were underlain by an increase in pressure or volume load on the heart. It was further established that the plasma levels of CT-ANF were, in general, proportional to the degree and duration of this overload. Thus the highest circulating plasma ANF levels were associated with long-standing essential hypertension and in chronic congestive heart failure classes III and IV. A similar historical development may be found for BNP, for which the development of a RIA demonstrated that elevated levels of circulating BNP were an indication of long-standing pressure or volume overload. Further, in the case of BNP a strong induction of the gene expression for these peptides was observed in the hypertrophied ventricle resulting in a relatively larger elevation of plasma BNP levels than those of ANF although, in absolute terms, circulating levels of ANF remain larger than those of BNP.

Having been established that important clinical entities are accompanied by changes in ANF and BNP gene expression, several clinical investigators have explored the possibility that ANF and BNP plasma levels may be used as diagnostic or prognostic indicators of cardiovascular disease. ANF plasma levels were used to establish long term prognosis after myocardial infarction MI(12), to stratify patients in terms of response to ACE inhibition post MI(23) and to demonstrate asymptomatic left ventricular dysfunction(1,15). Richards et al.(25) found a good correlation between plasma CT-ANF and cardiac output in elderly patients and further showed that plasma CT-ANF was a prognostic indicator of those who would subsequently develop chronic heart failure. Similarly, Davis et al.(6) showed that in the elderly, CT-ANF plasma levels identifies subjects at risk of CHF allowing for appropriate focussing of medical resources for the prevention, early detection and treatment of this syndrome. At the Mayo Clinic, Lerman et al.(15) have shown that NT-ANF is a highly sensitive marker for symptomless left ventricular dysfunction. Motvany et al(23) have shown that CT-BNP plasma levels identify patients with left ventricular dysfunction who have been identified by the SAVE study as likely to benefit from long term ACE treatment after MI. In this study it was found that plasma CT-ANF was not as predictive as CT-BNP. The usefulness of NT-ANF plasma levels to evaluate clinical status was re-emphasized by a study of Dickstein et al(10) who showed that NT-ANF correlated better than other variables with New York Heart Association functional class and was more closely associated with non invasive measurements than New York Heart Association functional class. Odds ratio measurement demonstrated substantially increased risk of left ventricular dysfunction and dilatation, pulmonary hypertension and New York Heart Association functional class 3 or 4 with increasing NT-ANF value. The

authors concluded that the data clearly indicated that the concentration of proANF is related to the degree of clinical heart failure. Moe et al(22) used NT-ANF to characterize hormonal activation in severe heart failure patients treated with flosequinan and showed a significant decline in plasma NT-ANF levels was observed in the survivors only. The importance of NP plasma level measurement was re-emphasized by Hall et al.(12) who, reporting for the Thrombolysis in Myocardial Infarction II investigators, showed that NT-ANF, when measured during the first 12 h after the onset of chest pain, is related to 1-year mortality after MI.

Of late, much of the attention directed towards the clinical measurement of NP in blood has been focused on CT- and NT-BNP. Some of this interest is based on the fact companies such as Roche, Biomedica and Biosite have put in the hands of researchers tests in a clinical kit format. Further, the development of these tests by industry is related to patent positions either in terms of peptide sequence or applications. At any rate, the results obtained are just as promising as those mentioned earlier that were carried out using RIAs. Maisel et al.(3,4,14,18,20) have evaluated the usefulness of rapid BNP tests measurements and have concluded that these tests are excellent screening tools to screen for LV systolic or diastolic dysfunction and may preclude the need for echocardiography in many patients. The diagnosis and titration of therapies in CHF and in the prediction of outcomes in patients admitted for decompensated heart failure. The prognostic value of BNP in acute coronary syndromes was demonstrated by De Lemos et al.(9) who concluded that a single measurement of BNP within a few days of the onset of ischemic symptoms predicts and risk stratifies across the spectrum of acute coronary syndromes including myocardial infarction with and without ST-segment elevation and unstable angina. Talwar et al.(19) examined the optimum time of blood sampling for NT-

BNP determination following MI and given that they found a biphasic pattern, they concluded that NT-BNP plasma level was a better predictor of poor outcome when measured later during hospitalisation than inmediately after MI.

It is to be noted that the studies that have compared measurements of NP with other neuroendocrine variables such as norepinephrine circulating levels, ANF and BNP have shown the closest correlation to cardiovascular functional status (10,13). It is tempting to speculate that in the early stages of heart failure, plasma renin activity and sympathetic activity are normal because the increased circulating levels of NP suppress them. This might explain why NP are elevated before other neuroendocrine variables and why the determination of NP plasma levels are an early and sensitive indicator of ventricular dysfunction.

It is also important to note the changes in NP secretion, as for example in studies demonstrating an elevation of plasma NP in subclinical systolic or diastolic dysfunction(1), are distinctive enough to make the measurements useful in diagnosis and prognosis beyond the group statistics, that is, they are useful adjuncts in the evaluation of the individual patient.

Finally, an exciting new application for the determination of NP plasma levels is the use of these determination of optimal therapies in heart failure. Troughton et al. (27) hypothesized that since there is no objective practical guide for the intensity of treatment in heart failure, they made a comparison of outcomes following either classical clinical criteria with plasma BNP-guided therapy. They found that BNP-guided treatment of heart failure reduced total cardiovascular events, and delayed time to first event compared with intensive clinically guided treatment.

Critical Appraisal of the Measurement of Natriuretic Peptide Plasma Levels within a Clinical Environment

Most of the studies described above were carried out using RIAs (RIA) to establish plasma levels of the different peptides.   RIAs can measure substances in the picoM to femtoM (10^{-12} - 10^{-15} M) range. This exquisite sensitivity though, implies slow turnaround times and requires highly trained personnel that routinely carries out this methodology and that is able to maintain a strict quality control program. In addition, the need to carefully and promptly handle blood specimens at 4 ·C and the need for highly specific antibodies, radioactivity, pre-purification from plasma and complex instruments such as gamma counters, makes RIAs not only expensive but nearly impossible to establish in other than tertiary care facilities and then, this is often associated with research laboratories only, where routine specimen collection outside normal working hours is more often than not, out of the question.

It is possible to measure at least 4 NP fragments: NT-ANF, CT-ANF, NT-BNP and CT-BNP (Fig. 1). The first

consideration about these possibilities is that no study has been conducted to compare head to head the relative usefulness of all four of these peptides at the same time and in a single population in diagnostic or prognostic terms. Recently, Talwar et al(26) compared the sensitivity, specificity and positive and negative predictive value of NP for identifying heart failure or LVSD. However this comparison was carried using data from different studies, which included patient populations with different inclusion criteria.

The NT- fragments of ANF, and that of BNP have much longer half lives than the biologically active CT- portions and, therefore, are potentially more useful for a clinical setting because: a. The plasma levels are the products of events integrated over a period of time thus providing more information about cardiovascular status than the CT-portions that have half-lives of the order of 5-20 min. b. The plasma levels of the NT-portion of ANF and BNP are much higher than those of CT-portions allowing for the development of technology that are less exacting and far less expensive than RIA. c. It has been reported that RIA (11) for NT-ANF can be carried out without previous
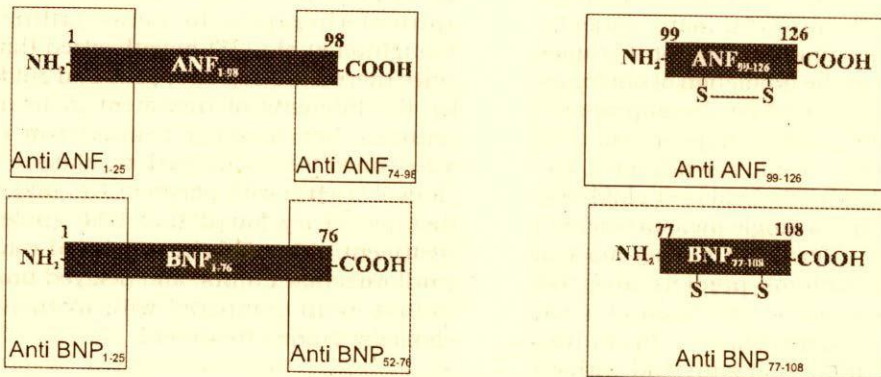

Figure 1. Circulating forms of ANF and BNP. Polyclonal antibodies and RIAs available are indicated by the rectangles

extractions and in blood samples that were collected and left purposely at room temperature for extended periods of time. A similar finding was reported in a short note for CT-BNP(5) (surprising given the short half life of this peptide in plasma). These findings, however, have not been widely confirmed and many laboratories carrying out NT-ANF determinations in plasma still extract and treat specimens with special precautions.

Finally, we recently(21) reported the potential of measurement of BNP in blood for the detection and prediction of acute cardiac allograft rejection. It was found that all rejection episodes receiving treatment were accompanied by a marked increase in BNP plasma to levels greater than about 400 pg/mL. Steadily increasing BNP levels preceded overt rejection as assessed by histopathological criteria. Treatment of the acute rejection episodes led to a substantial decrease of BNP plasma levels.

In summary, the measurement of NP levels in cardiac disease has resulted in an improvement in several areas of care for the cardiac patientthe cardiac patients, in particular those afflicted with congestive heart failure since it is now possible to objectively diagnose it. Perhaps the biggest value of NP plasma values as an inexpensive screening method is its ability to discriminate between symptoms arising from heart failure and those arising from other sources like lung pathologies.

REFERENCES

1. Arad M, Elazar E, Shotan A, Klein R and Rabinowits B. Brain and atrial natriuretic peptides in patients with ischemic heart disease with and without heart failure. *Cardiology* 87: 12-17, 1996.

2. Bryan WM. Design of minimum active fragments of biologically active peptides. *Methods In Enzymology* 202: 436-448, 1991.

3. Cheng V, Kazanagra R, Garcia A, Lenert L, Krishnaswamy P, Gardetto N, Clopton P and Maisel A. A rapid bedside test for B-type peptide predicts treatment outcomes in patients admitted for decompensated heart failure: a pilot study. *J Am Coll Cardiol* 37: 386-391, 2001.

4. Dao Q, Krishnaswamy P, Kazanegra R, Harrison A, Amirnovin R, Lenert L, Clopton P, Alberto J, Hlavin P and Maisel AS. Utility of B-type natriuretic peptide in the diagnosis of congestive heart failure in an urgent-care setting. *J Am Coll Cardiol* 37: 379-385, 2001.

5. Davidson NC, Coutie WJ and Struthers AD. N-terminal proatrial natriuretic peptide and brain natriuretic peptide are stable for up to 6 hours in whole blood in vitro [letter]. *Circulation* 91: 1276-1277, 1995.

6. Davis KM, Fish LC, Elahi D, Clark BA and Minaker KL. Atrial natriuretic peptide levels in the prediction of congestive heart failure risk in frail elderly. *JAMA* 267: 2625-2629, 1992.

7. de Bold AJ. Atrial natriuretic factor: a hormone produced by the heart. *Science* 230: 767-770, 1985.

8. de Bold AJ, Borenstein HB, Veress AT and Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. *Life Sci* 28: 89-94, 1981.

9. de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, Hall C, Cannon CP and Braunwald E. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med* 345: 1014-1021, 2001.

10. Dickstein K, Larsen AI, Bonarjee V, Thoresen M, Aarsland T and Hall C. Plasma proatrial natriuretic factor is predictive of clinical status in patients with congestive heart failure. *Am J Cardiol* 76: 679-683, 1995.

11. Hall C, Aaberge L and Stokke O. In vitro stability of N-terminal proatrial natriuretic factor in unfrozen samples: an important prerequisite for its use as a biochemical parameter of atrial

pressure in clinical routine [letter]. *Circulation* 91: 911, 1995.

12. Hall C, Cannon CP, Forman S and Braunwald E. Prognostic value of N-terminal proatrial natriuretic factor plasma levels measured within the first 12 hours after myocardial infarction. *J Am Coll Cardiol* 26(6): 1452-1456, 1995.

13. Hall C, Rouleau JL, Moyé L, de Champlain J, Bichet D, Klein M, Sussex B, Packer M, Rouleau J, Arnold MO, Lamas GA, Sestier F, Gottlieb SS, Wun C-CC and Pfeffer MA. N-Terminal Proatrial Natriuretic Factor. *Circulation* 89: 1934-1942, 1994.

14. Kazanegra R, Cheng V, Garcia A, Krishnaswamy P, Gardetto N, Clopton P and Maisel A. A rapid test for B-type natriuretic peptide correlates with falling wedge pressures in patients treated for decompensated heart failure: a pilot study. *J Card Fail* 7: 21-29, 2001.

15. Lerman A, Gibbons RJ, Rodeheffer RJ, Bailey KR, McKinley LJ, Heublein DM and Burnett JC, Jr. Circulating N-terminal atrial natriuretic peptide as a marker for symptomless left-ventricular dysfunction [see comments]. *Lancet* 341: 1105-1109, 1993.

16. Lincoln TM and Cornwell TL. Intracellular cyclic GMP receptor proteins. *FASEB J* 7: 328-338, 1993.

17. Maekawa K, Sudoh T, Furusawa M, Minamino N, Kangawa K, Ohkubo H, Nakanishi S and Matsuo H. Cloning and sequence analysis of cDNA encoding a precursor for porcine brain natriuretic peptide. *Biochem Biophys Res Commun* 157: 410-416, 1988.

18. Maisel A. B-type natriuretic peptide levels: a potential novel "white count" for congestive heart failure. *J Card Fail* 7: 183-193, 2001.

19. Maisel AS. Practical approaches to treating patients with acute decompensated heart failure. *J Card Fail* 7: 13-17, 2001.

20. Maisel AS. Practical approaches to treating patients with acute decompensated heart failure. *J Card Fail* 7: 13-17, 2001.

21. Masters RG, Davies RA, Veinot JP, Hendry PJ, Smith SJ and de Bold AJ. Discoordinate Modulation of Natriuretic Peptides During Acute Cardiac Allograft Rejection in Humans. *Circulation* 100: 287-291, 1999.

22. Moe GW, Rouleau JL, Charbonneau L, Proulx G, Arnold JM, Hall C, de Champlain J, Barr A, Sirois P and Packer M. Neurohormonal activation in severe heart failure: relations to patient death and the effect of treatment with flosequinan. *Am Heart J 2000 Apr ;139 (4):587 -95* 139: 587-595, 2000.

23. Motwani JG, McAlpine H, Kennedy N and Struthers AD. Plasma brain natriuretic peptide as an indicator for angiotensin- converting-enzyme inhibition after myocardial infarction. *Lancet* 341: 1109-1113, 1993.

24. Murakami Y, Shimada T, Inoue S, Shimizu H, Ohta Y, Katoh H, Nakamura K and Ishibashi Y. New insights into the mechanism of the elevation of plasma brain natriuretic polypeptide levels in patients with left ventricular hypertrophy. *Can J Cardiol* 18: 1294-1300, 2002.

25. Richards AM, Crozier IG, Yandle TG, Espiner EA, Ikram H and Nicholls MG. Brain natriuretic factor: regional plasma concentrations and correlations with haemodynamic state in cardiac disease. *Br Heart J* 69: 414-417, 1993.

26. Talwar S, Downie PF, Ng LL and Squire IB. Towards a blood test for heart failure: the potential use of circulating natriuretic peptides. *Br J Clin Pharmacol* 50: 15-20, 2000.

27. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG and Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 355: 1126-1130, 2000.

28. Yokota N, Bruneau BG, Fernandez BE, Kuroski de Bold ML, Piazza LA, Eid H and de Bold AJ. Dissociation of cardiac hypertrophy, myosin heavy chain isoform expression, and natriuretic peptide production in DOCA-salt rats. *Am J Hypertens* 8: 301-310, 1995.