Are natriuretic peptides clinically useful as markers of heart failure?

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Heart failure is a clinical syndrome of signs and symptoms caused by left ventricular dysfunction. It is the most serious expression of heart disease and encompasses a wide spectrum of pathophysiological states, ranging from those caused by sudden impairment of pump function (e.g. massive myocardial infarction) to the progressive and gradual impairment of myocardial function, for example in patients whose heart is subjected to pressure or volume overload, or who have a chronic disorder of the heart muscle. In practical terms heart failure is diagnosed when symptoms of breathlessness (at rest or on exertion) or fatigue and signs of fluid retention (peripheral oedema, pulmonary crepitations or elevated jugular venous pressure) are found in a patient already suspected of having heart disease. Cardiac dysfunction is an essential element in the diagnosis of heart failure. The commonest cause is left ventricular systolic dysfunction (LVSD) due to myocardial disease. The syndrome of heart failure is primarily cardiac, but secondary multisystem dysfunction often follows, leading to a terminal state of multiorgan failure.

PHYSIOLOGY AND BIOCHEMISTRY OF NATRIURETIC PEPTIDES

There are three natriuretic peptides (NP): atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), the precursor prohormone for each of which is encoded by a separate gene. The tissue-specific distribution and regulation of each peptide is different.

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ANP is produced mainly in the atria. Increased atrial wall tension, reflecting increased intravascular volume, is the dominant stimulus for its release. The mRNA transcript for ANP is approximately 1 kb in size and encodes a precursor protein (pro-ANP) of 126 amino acids. Cleavage of human pro-ANP releases a 98-amino acid amino-terminal fragment (N-ANP), as well as a 28-amino acid carboxy-terminal fragment that is mature ANP. Both fragments circulate in the plasma and their concentrations are increased in patients with increased intravascular volume, e.g. heart failure. Very little ANP is produced by ventricular tissue in normal adults, but it is present in the ventricular tissue of foetuses and neonates, and in hypertrophied and failing ventricles.[1,2] The ANP gene is also expressed in the kidney, where alternative processing of the precursor generates a 32-amino acid substance, urodilatin, one of the factors that regulate sodium and water handling in the kidney.[3]

BNP was originally found in extracts of porcine brain. It is present in human brain and is more abundant in the cardiac ventricles. Human pro-BNP contains 108 amino acids; processing releases a mature 32-amino acid molecule and an amino-terminal fragment (N-BNP or N-terminal proBNP). Both circulate in plasma and the concentrations are high in heart failure and in ventricular hypertrophy.

CNP exists in a 22- and a 53-amino acid form. Each is derived from the pro-CNP precursor through a different process, and the 22-amino acid form is included in the carboxy-terminus of the 53-amino acid form. The 22-amino acid peptide predominates in the brain, anterior pituitary, kidney, vascular endothelial cells and plasma, and is more potent than the 53-amino acid form. The serum concentration of CNP is very low.

NP receptors

NPs bind to high-affinity receptors on the surface of target cells. Three types exist: A, B

and C. A and B are linked to the cyclic guanosine monophosphate (cGMP)-dependent signalling cascade and mediate the cardiovascular and renal effects of natriuretic peptides. The A receptor binds ANP and BNP, with preference for ANP. The B receptor binds CNP. The A receptor is the most abundant receptor in the blood vessels. Both receptors are also present in the adrenal glands and kidneys. Receptor C is involved in clearance of the peptides.[4,5] The three NPs bind to the C receptor with equal affinity. NPs are also inactivated by neutral endopeptidases found in renal tubular cells and vascular cells. The relative contribution of each system is not yet known.

Cardiovascular physiology of NPs

ANP increases venous capacitance and promotes natriuresis, reducing extracellular fluid volume by its effect on the kidney.[6] In the peripheral vasculature ANP reduces sympathetic tone by dampening baroreceptors, by suppressing catecholamine release from autonomic nerve endings, and by suppressing sympathetic outflow from the central nervous system.[7,8] ANP lowers the activation threshold of vagal afferents, suppressing the reflex tachycardia and vasoconstriction that accompany the reduction in cardiac preload (right heart filling pressure and venous return) and ensuring a sustained decrease in mean arterial pressure.

BNP has similar cardiac effects to ANP. CNP is a more potent dilator of veins than the other two peptides. CNP is found in human brain, vascular endothelium and kidney, and in humans seems to act as a local regulator or neuropeptide rather than as a cardiac hormone. Each NP also has antimitogenic activity. ANP and CNP inhibit mitogenesis in cultured vascular cells and in balloon-injured carotid arteries in rats.[9,10] This suggests that natriuretic peptides may modulate growth within the vascular wall in processes such as atherosclerosis, hypertension and post-angioplasty restenosis.

Renal physiology of NPs

ANP stimulates the dilatation of afferent renal arterioles and the constriction of efferent arterioles, leading to increased pressure in glomerular capillaries.[11] This causes an increase in glomerular filtration. The peptide also increases the accumulation of cGMP in mesangial cells, which relaxes these cells and thereby increases the effective surface area for filtration. Plasma concentrations of ANP that do not increase the glomerular filtration cause natriuresis, indicating that the peptide has direct tubular actions. ANP inhibits sodium transport in the collecting duct, decreases renin secretion from the macula densa, inhibits aldosterone release from the zona glomerulosa, and inhibits tubular water transport by antagonizing the action of vasopressin. In the inner medullary collecting duct ANP stimulates cGMP production and blocks sodium absorption.[12] In humans, BNP infusions also reduce plasma renin and aldosterone and inhibit angiotensin II-stimulated aldosterone secretion.[13] CNP has little effect. In diabetes and in patients with ascites due to liver cirrhosis, abnormalities in NP metabolism may be caused by blunted renal responsiveness to NP in the early stages of these diseases.[14,15] In addition, it has been shown that NP antagonists, e.g. HS-142-1, given to animals with experimentally induced heart failure, block NP-induced natriuresis and diuresis, increase renal vascular resistance, and increase plasma renin, aldosterone and catecholamine concentrations. In diabetic or cirrhotic rats with ascites this NP antagonist reduces renal plasma flow and glomerular filtration, suggesting a beneficial compensatory response for NPs in ascites.[16]

Central nervous system physiology of NPs

ANP and BNP do not cross the blood–brain barrier but they do reach sites nearby (i.e. subfornical organ and hypothalamus). All three NPs are produced in the brain. Endothelin, noradrenaline and arginine vasopressin (AVP) stimulate the release of ANP from hypothalamic neurons *in vitro*.[17,18] The actions of NPs in the brain reinforce those in the periphery. In the brain stem NPs decrease sympathetic tone.[19] In rats, ANP regulates cardiovascular baroreceptor signalling to the nucleus tractus solitarius. The NP actions in the central nervous system are best explained by the presence of A- and C-type receptors. C receptors are extensively distributed throughout the brain, whereas A receptors are found in areas adjacent to the third ventricle that are not separated from the blood by the blood–brain barrier, a position that allows binding of circulating ANP as well as of locally produced peptide.[20] This receptor appears to regulate the effects of ANP on salt appetite and water drinking. BNP receptors predominate in the hypothalamus, where the peptide inhibits the secretion of AVP and paradoxically stimulates sympathetic tone.

ASSAY OF PLASMA NP

Plasma ANP concentration

Venous blood samples are collected into chilled tubes containing EDTA (potassium salt) and 4000 kallikrein inhibitory units of aprotonin (Trasylol) as a preservative. Plasma is separated by centrifugation at 4°C for 15 minutes at 3000 rpm and stored at −70°C until assay. The samples for each individual are assayed in batches.

ANP is initially extracted from plasma over C-18 silica columns,[21] the extraction recovery in our laboratory being 88·3%. ANP is then measured by immunoassay.[21] The reference range for supine ANP in our laboratory is 2·4–10·5 pmol/L, and our inter-assay coefficient of variation (CV) is 12·6%.

Plasma BNP concentration

Samples for BNP are collected, extracted and assayed as for ANP, except that an antibody specific for BNP is used. Recovery following extraction in our laboratory is 85·9%, our reference range is 2·3–4·5 pmol/L, and our inter-assay CV is 9·9%.

The analytical limitations of the BNP assay are discussed later.

USE OF NATRIURETIC PEPTIDES AS MARKERS OF HEART FAILURE

ANP – diagnostic use

In symptomatic congestive heart failure both plasma C-terminal and N-ANP concentrations are increased. N-ANP concentrations are increased in asymptomatic left ventricular dysfunction (LVD) (N-ANP has 90% sensitivity and 92% specificity).[22] N-terminal ANP may also be used to identify patients with symptomatic LVSD (sensitivity 89%, negative predictive value 92%).[23] Plasma ANP concentrations apparently correlate with the left ventricular ejection fraction (LVEF) and with the severity of heart failure.[24]

ANP – prognostic use

A substudy of SAVE (Survival and Ventricular Enlargement Study 1992),[25] concluded that N-ANP was a powerful predictor of long-term mortality in post-myocardial infarction (MI) patients independent of any of the other neurohormonal or haemodynamic parameters that were measured, including the LVEF.[26] In patients with diastolic and systolic LVD [ejection fraction (EF) <50%; normal value >55%] ANP concentrations are higher in those with a restrictive filling pattern and may indicate more severe heart failure.[27] ANP is also a marker of morbidity in heart failure patients, and a moderate elevation in N-ANP concentrations is associated with a substantially prolonged hospital stay.[28]

BNP – diagnostic use

BNP is elevated in post-MI patients with impaired LV function (EF<40%).[29] It appears to be superior to N-ANP for identifying LVSD. In our own head-to-head comparison the sensitivity and specificity of BNP for identifying LVSD (EF<35%) were 100% and 58%, respectively, whereas those for N-ANP were 95% and 35%, respectively.[30] Similar studies have been performed by other groups, but most have selected patients undergoing cardiac investigations in a hospital setting.[31–34] Such a population may be different from the kind of general practice population where plasma NP concentrations would be most useful. In the Hillingdon study, in a general practice setting, plasma BNP had 97% sensitivity and 84% specificity for identifying heart failure accurately.[35] Comparable figures for N-ANP were 97% and 66%, respectively.[35]

In the Glasgow study of LVD (EF<35%) in the general population, BNP was elevated in both symptomatic and asymptomatic ventricular dysfunction, as was N-ANP. BNP had 77% sensitivity and 87% specificity in all individuals. In patients over 55 years of age with ischaemic heart disease, the sensitivity and specificity increased to 92% and 72%, respectively.[36] In elderly patients BNP remains a sensitive marker of impaired LV function after MI, in dilated cardiomyopathy and in valvular heart disease.[34] Combining N-ANP with BNP does not add to the predictive value over using BNP alone. The very high negative predictive value of BNP in the detection of LVSD gives the best insight into how this test might be used clinically.

The superiority of BNP over ANP for identifying LVD may be explained by the fact that BNP is derived predominantly from ventricular tissue whereas ANP is from atrial tissue in normal subjects, although in heart failure ANP is probably derived from ventricular tissue as well. BNP secretion is significantly greater from infarcted tissue than from the non-infarcted region of the left ventricle.[37] Increased regional wall stress is believed to be associated with adverse ventricular remodelling and a poor

prognosis after MI.[25] A potential association between plasma BNP concentration and LV remodelling may contribute to the independent prognostic value of BNP.

BNP – prognostic use

BNP is a very useful prognostic marker. In chronic heart failure BNP predicts mortality.[38–43] It also predicts ventricular dysfunction and survival after MI.[42] Elevated BNP increases the risk of sudden death after MI and may indicate early left ventricular remodelling in these patients.[40] In Darbar's study[40] BNP was a better predictor of future LVD (EF < 40%) than was the ejection fraction measured immediately after the acute MI. This could be because risk factors other than the degree of systolic dysfunction might also affect the release of NPs, i.e. NP concentration may be a composite result of several adverse cardiac features, rather than just one. This could explain why NPs perform better as prognostic indicators than as diagnostic indicators. Additional factors of prognostic significance may include the presence of diastolic dysfunction, the presence of left ventricular hypertrophy and asymmetry, or the general level of neuroendocrine activation. For example, the release of both ANP and BNP is stimulated in cultured cells by endothelins, which circulate at increased concentrations in the plasma of patients with heart failure.[45] Endothelins, like NPs, are a good indicator of prognosis.[46] However recent studies suggest that BNP is a better marker of prognosis in heart failure than are endothelins.

N-terminal pro-BNP (N-BNP)

BNP may be assayed as N-BNP or as intact BNP. Assay of intact BNP may be as sensitive as echocardiography and superior to clinical examination.[47] Cut-off values for plasma concentrations of BNP have been established in screening for LVSD (EF < 35%) in the general population, in patients with chronic heart failure, and after an acute myocardial infarction.[30,36] Unfortunately, direct head-to-head comparisons with N-BNP assays are not available. The 76-amino acid N-BNP circulates in plasma in higher concentrations than does intact BNP in patients with heart failure and in asymptomatic LVD. Plasma N-BNP measured 2–4 days after acute myocardial infarction predicts left ventricular function and 2-year survival (sensitivity 85%, negative predictive value 91%).[48] Talwar *et al.*[49] have recently shown that N-BNP > 275 pmol/L predicted LVSD (Wall motion index < 1·2) with a sensitivity of 93·8%, a specificity of 55% and a negative predictive value of 93%. An interesting finding of this study was the reliability of the N-BNP assay for predicting LVSD even in patients who had renal dysfunction and in patients taking medication, as concerns had been expressed that angiotensin-converting enzyme (ACE) inhibitor therapy, diuretics, β-blockers and digoxin might modify plasma concentrations of NPs and nullify their potential as markers for left ventricular dysfunction.[50–53] The negative predictive value of N-BNP compares very favourably with intact BNP assays (97%, 98%). The advantage of the former is that its plasma concentration is 10 times as high as that of BNP, which potentially makes it easier to devise a stix test for bedside testing. However, to date only stix tests for BNP[54] and not for N-BNP are commercially available. In Richards' study[48] N-BNP and intact BNP were equally sensitive and specific (71%, 68% for sensitivities; 69%, 69% for specificities) and the negative predictive value for identifying patients after MI having an LV ejection fraction of < 40% was 80% for N-BNP and 79% for intact BNP. In fact, N-BNP concentrations five times (70 pmol/L) and intact BNP concentrations twice (20 pmol/L) the upper limit of normal retained both sensitivity and negative predictive values of ⩾ 90% for LVEF < 40%. N-BNP was superior to intact BNP for predicting mortality and heart failure during the 2 years after myocardial infarction.[48]

COMPARISON OF NPs WITH OTHER MARKERS FOR HEART FAILURE

NPs identify patients with symptomatic (heart failure) and asymptomatic LVSD. However, in asymptomatic LVSD, although these peptides are very sensitive they lack specificity, as BNP may also be elevated in any form of cardiac disease. Echocardiography (ECh) is therefore required to identify the underlying cardiac pathology. NP measurement should be used to select patients for ECh but cannot substitute for ECh, which will reveal the systolic and diastolic ventricular function and thus determine the appropriate treatment. In turn, plasma BNP may be used to assess the therapeutic response to drugs such as ACE inhibitors.[55] Another important point is that prognosis is determined by systolic and diastolic dysfunction, whereas treatment is currently determined only by the

presence of systolic dysfunction.[56] BNP concentrations are elevated in both systolic and diastolic dysfunction, which is probably why BNP is a better marker of prognosis than it is of diagnosis.[57]

Comparison with signs and symptoms

As previously mentioned, signs and symptoms correlate poorly with the presence of heart failure. BNP measurement may, however, exclude normal hearts and therefore reduce the ECh burden. Recently, it has been shown that if the ratio of heart rate to diastolic blood pressure is >1, there is LVD (sensitivity 86%, negative predictive value 93%).[23] When such a ratio was found together with an abnormal ECG, LVD was even more likely to be detected on ECh.[23] In this study, ANP was superior to these clinical markers of LVSD ($EF<40\%$) (sensitivity 89%, negative predictive value 92%).[23] Plasma BNP was not assessed. However, it has been shown by our department[47] in post-MI patients that plasma BNP is superior to all clinical indices of LVSD, including signs and symptoms and modified clinical scoring tests (Peel Index) (sensitivity 84% versus 46–64%), and that plasma BNP was a better predictor of LVD ($EF<40\%$) than was plasma ANP (sensitivity 84% versus 64%).[47]

Comparison with 12-lead ECG

In the Edinburgh study,[58] Davie *et al.* found that LVSD was virtually never present if the ECG was normal (sensitivity 94%, negative predictive value 98%), and a screening ECG reduced the number of echocardiograms required by 50%. Whereas the sensitivity of an abnormal ECG exceeds that of NP measurement, in other studies the success of combining the two in predicting ventricular dysfunction is variable. In Talwar's group, N-BNP plus an abnormal ECG was not superior to BNP alone for identifying LVD.[49] However, the sensitivity and specificity of an abnormal ECG for identifying patients with LVD were both very low, which is inconsistent with previously reported results, including our own. In our study[59] of LVSD ($EF<40\%$) in vascular patients (i.e. those presenting after their first stroke, first transient ischaemic attack, or first presentation with overt peripheral vascular disease) we found that an abnormal ECG identifies 81% of LVSD (sensitivity 81%, negative predictive value 86%). When we combined BNP measurement with an abnormal ECG, the specificity improved from 48% to 62%. In patients with the most severe LVD ($EF<30\%$) BNP and an abnormal ECG identified all patients (sensitivity 100%, specificity 56%), whereas an abnormal ECG alone detected only 90% of patients with LVSD. In Nielsen's study[23] N-ANP combined with an abnormal ECG also increased the likelihood of LVSD detection.

Comparison with chest X-ray

Radiographic cardiomegaly (enlarged heart on chest X-ray) is 51% sensitive and 79% specific for heart failure.[60] Comparing NP measurement with radiographic cardiomegaly, Cowie *et al.*[35] found that the area under the receiver operating characteristic curve for BNP was 0·96, whereas for radiographic cardiomegaly (cardiothoracic ratio) it was 0·76. This suggests that BNP combined greater sensitivity and higher specificity over a range of different cut-off values than did cardiothoracic ratios or other NP concentrations. In subsequent logistic regression analysis including both cardiothoracic ratio and BNP, only the BNP concentration was independently predictive of the presence of heart failure.

It is apparent that no single test is the best for screening patients with suspected heart failure; it may well be that a combination of tests is the optimal approach, both to screening and to risk-stratifying suspected heart failure patients. Indeed, a recent New Zealand study[48] showed that a combination of plasma BNP and ECh assessment of LV function better defined the risk of mortality and/or heart failure in post-MI patients than did either test alone.

Limitations of BNP assay

The difficulty with using plasma BNP as a guide to diagnosis or as a prognostic indicator is that a moderate degree of elevation lacks specificity. MI, left ventricular hypertrophy (LVH), cardiomyopathy, cardiac failure, renal failure and chronic obstructive airways disease can all increase plasma BNP. In a study[61] of patients with suspected heart disease, a plasma BNP $>15·7$ pmol/L was 85% sensitive and 74% specific for detecting LV diastolic dysfunction and 81% sensitive and 90% specific for identifying LVH (increased LV mass). In a more recent study[62] N-BNP was shown to detect LVSD (Wall motion index $<1·2$) independent of the presence of hypertension or indeed LVH ($P<0·0001$). A high plasma BNP calls for further investigation; a normal or low BNP has

excellent negative predictive value for heart failure and may obviate the need for further costly studies, including ECh.

A further limitation with BNP assays that must be recognized is that concomitant cardiac medications may affect the diagnostic accuracy of the BNP assay: recent studies have shown that frusemide reduces NP concentrations[51] without affecting the echocardiogram, thus producing a false-negative NP result in the presence of LVSD. NPs should therefore be measured before the introduction of diuretic therapy. In contrast, β-blockers seem to increase BNP while having a beneficial effect on LV function.[52]

CONTROVERSIES ABOUT BNP ASSAY

Controversies surround the BNP plasma assay. Initial studies suggested that BNP was unstable, with a 30–40% reduction of BNP after 3 h of storage.[63] However, two more recent studies[64,65] showed that BNP is stable in whole blood for up to 3 days. In addition, BNP assays have become more precise since assays not requiring extraction have been developed.[64] A wide variety of plasma ANP concentrations in healthy individuals was reported earlier which may relate to the extraction process, especially if it is preceded by acidification. Most immunoassays for measuring BNP are also based on extraction with solid-phase resin. Plasma concentrations of immunoreactive BNP in normal subjects are lower than those of ANP. A high molecular mass precursor (?pro-BNP) and the low molecular mass BNP have been detected in human heart and plasma. This contrasts with ANP, where the major form in human atrial tissue is the full pro-ANP. The presence of the two BNP forms, both of which are measured by most current immunoassays, raises the issue of specificity but, given the strong correlation between the plasma concentrations of the two forms, total immunoreactive plasma BNP remains an index of augmented synthesis and secretion.[64] Arguments for using extracted rather than non-extracted assays are unclear at present, and methods for measuring NP are now moving towards the use of stix tests directly on blood samples.[54]

The choice of which NP to measure is simplified by the fact that raised concentrations of ANP and BNP in patients with cardiovascular disease are usually also associated with higher concentrations of the corresponding precursors. However, it is also apparent that differences in pathophysiology and/or elimination rates may lead to subtle differences in the relative proportions of these peptides in plasma. Current work suggests that BNP and N-BNP are superior to N-ANP and ANP for assessing ventricular dysfunction and predicting the mortality outcome in those with severe heart failure.

CONCLUSION

A normal BNP virtually excludes LVSD and hence heart failure; a high BNP concentration merely indicates the presence of cardiac disease and the need for an echocardiogram (which requires expensive equipment, highly trained staff and subjective interpretation) to determine whether the problem is LVSD, LV diastolic dysfunction or both, or even valve disease. As BNP is a marker of both systolic and diastolic ventricular dysfunction it is a useful prognostic marker, especially in identifying high-risk heart failure patients and in asymptomatic left ventricular dysfunction after MI. This may help to monitor the effects of treatment or to prioritize interventions such as cardiac transplantation. A recent study from Glasgow[55] showed that plasma BNP estimation can be used to tailor vasodilator therapy with ACE inhibitors in heart failure patients without having to use invasive measurements such as Swan–Ganz catheterization. In addition, Troughton *et al.*[67] have showed that using BNP concentrations to guide treatment of patients with LVD (EF < 40%) and symptomatic heart failure reduced total cardiovascular events (death, hospitalization or heart failure decompensation) ($P = 0.02$) and delayed the time to first cardiovascular event ($P = 0{\cdot}034$) compared to intensive clinically guided treatment. BNP has also been used to assess the cardiotoxicity of chemotherapy, to monitor the severity of valvular heart disease and to assess the success of cardiac transplantation.[68–70]

In heart failure patients BNP is probably most useful as a marker of prognosis, to identify those at highest risk of mortality, recurrent hospitalization or further morbidity, so that they can be identified and treated as a priority.

Despite the merits of measuring plasma BNP there is still much controversy concerning the best assay method, permissible storage times and temperature. Only further investigation and

randomized trials will evaluate the clinical use of NPs as markers of heart failure, especially in the presence of cardiac medications such as β-blockers and diuretics.

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