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REVIEW

Brain Natriuretic Peptide: Structure, Action, and Role in the Diagnosis and Prognosis of Heart Failure

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Abbreviations
ACE = angiotensin converting enzyme
ANP = atrial natriuretic peptide
ARB = angiotensin II receptor blocker
ARNI = angiotensin receptor neprilysin inhibitor
BNP = brain-type natriuretic peptide
cGMP = cyclic guanosine monophosphate
CNP = C-type natriuretic peptide
ECG = electrocardiogram
MI = myocardial infarction
MMP = matrix metalloproteinase
NEP = neprilysin
NFAT = nuclear factor of activated T cells
NPR-A = natriuretic peptide receptor-A
NT-pro-BNP = N-terminal fragment of brain-type natriuretic peptide
PKG = protein kinase G

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ABSTRACT

Brain natriuretic peptide (BNP) is a peptide hormone secreted by cardiomyocytes in response to atrial or ventricular wall stretch and/or pressure overload. It promotes a number of systemic effects, including vasodilatation, increase in urinary output and sodium excretion, as well as inhibition of the sympathetic nervous system and the renin–angiotensin–aldosterone system. Plasma BNP levels have been reported to be elevated in patients with left ventricular hypertrophy, congestive heart failure, acute coronary syndromes, atrial fibrillation, and impaired renal function. Moreover, elevated BNP levels have been shown to be a strong predictor of morbidity and mortality in patients with heart failure. Interestingly, it has also been found that the N-terminal peptide of BNP is slightly superior to BNP for predicting death or re-hospitalization for heart failure. Presumably, it is the longer half-life of N-terminal fragment of brain-type natriuretic peptide (NT-pro-BNP) that may promote it as a more accurate index of ventricular stress and therefore a better predictor of prognosis.

INTRODUCTION

Over the past few decades, exciting advances have been made in the field of cardiac biomarkers and their key role as important tools for diagnosis, risk stratification and therapeutic decision-making in patients with suspected acute coronary syndromes and other cardiovascular disease. According to the World Health Organization, a biomarker is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[1] It has been concluded that a biomarker is accepted as clinically useful when the following criteria are fulfilled: i) availability of accurate and repeated measurements at a reasonable cost with short turnaround times, ii) provision of information that is not available from a thorough clinical examination and iii) assistance in clinical decision making.[2] Indeed, cardiac troponins T and I play an essential role for diagnostic work up in patients suffering from acute myocardial infarction (MI).

Currently, researchers are investigating several promising new biomarkers, with the brain-type natriuretic peptide (BNP) being one with proved diagnostic usefulness in a great number of studies, which has thus progressed from benchside to clinical

Conflict of Interest: none declared

application at the bedside. Specifically, BNP secreted by the cardiac ventricles has emerged as a novel biomarker for monitoring and prognosis of left ventricular dysfunction in hypertensive subjects. Besides, in recent studies, BNP has also been proposed as an independent marker for atrial fibrillation and cardioembolic stroke associated with poor clinical outcomes.[3] In this review we are going to summarize existing data concerning the structural characteristics, the mode of action and the clinical significance of BNP and its N-terminal fragment (NT-pro-BNP) in the diagnosis and prediction of heart failure. Furthermore, we will review data assessing the validity of this biomarker in screening asymptomatic subjects at risk for heart failure.

SYNTHESIS, ACTION, AND BNP CLEARANCE BY THE KIDNEYS

In recent years, researchers have isolated originally from porcine brain extracts a neurohormone named BNP, which along with atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) form a triple natriuretic peptide system of the heart muscle. All peptides share a similar amino-acid sequence homology. Heart failure, as well as renal insufficiency, constitute clinical conditions where the natriuretic peptide system can become activated.

It has been demonstrated that BNP is synthesized and secreted by the cardiomyocytes in response to increased myocardial wall stress. Data suggest that a significant amount of BNP is produced and released into the blood circulation by the human ventricles in contrast to ANP, which is mainly secreted by the atria, and is involved in fluid, electrolyte and vascular homeostasis.[4,5] Specifically, it has been shown that BNP is produced by ventricular cardiomyocytes in response to pressure overload in the left ventricle as an inactive prohormone, which is later cleaved by an enzyme called corin into the active hormone BNP and the inactive NT-pro-BNP[4] (Fig. 1).

Human BNP is produced as a 108 amino acid prohormone (pro-BNP-108), an inactive proform, which is converted to the biologically active peptide BNP32, and an inactive N-terminal (NT-pro-BNP) through cleavage by proteolytic enzymes. Previous studies have shown that both corin, a cardiac serine protease, and furin, a ubiquitous serine protease, are critical in mediating pro-BNP processing in cardiomyocytes.[6]

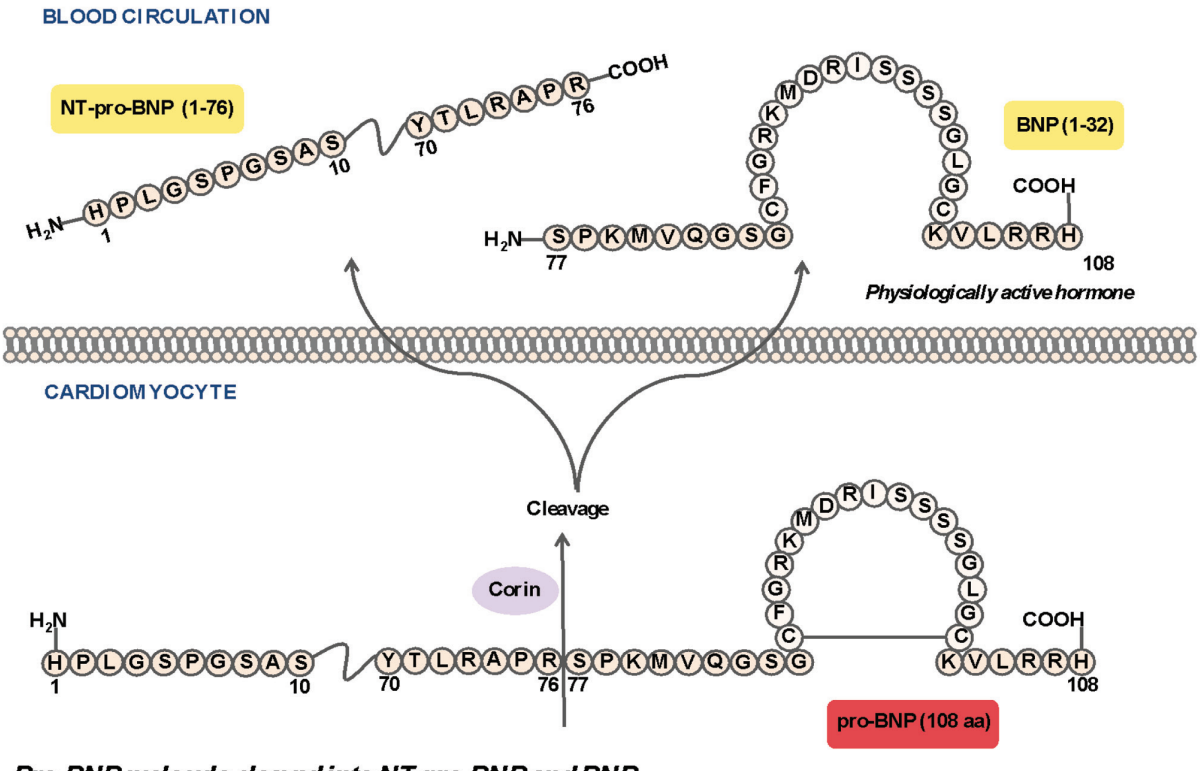


FIGURE 1. Human brain natriuretic peptide (BNP) is produced as a 108 amino acid prohormone (proBNP-108), an inactive proform, which is converted to the biologically active peptide BNP32, and an inactive N-terminal (NT)-pro-BNP through cleavage by a proteolytic enzyme, called corin.

As far as we know, corin consists of 1042 amino acids with an integral transmembrane domain near the N-terminal region, and is mainly expressed in the normal human heart and kidney, especially in cells around the vasculature. On the other hand, furin is expressed in the Golgi apparatus of many types of cells.[7] To gain insight into the sequence-specificity of furin- and corin- mediated pro-BNP processing, Semenov et al used non-glycosylated pro-BNP made in Escherichia Coli, and showed that furin cleaved pro-BNP at Arg-76, generating BNP1-32, whereas corin cleaved pro-BNP at Lys-79, generating BNP4-32.[8] Another study clearly demonstrated that in cardiomyocytes pro-BNP processing is mediated by the corin enzyme at several different sites. This differed significantly from BNP-processing in human embryonic kidney (HEK) 293 cells, where furin cleaved pro-BNP at a single residue (Arg-76). Interestingly, corin also activates ANP.[9] However, further studies are needed to provide insights into the biochemical basis of pro-BNP processing.

Following its secretion, BNP binds preferentially to the natriuretic peptide receptor-A (NPR-A) to exert its effect in the regulation of intravascular blood volume and vascular tone. Existing evidence suggests that BNP acts not only as a circulating hormone, but also as an autocrine and/or paracrine factor in order to exert its cardioprotective role against myocardial hypertrophy and fibrosis.[10] Specifically, NPR-A is linked to guanylyl cyclase, which upon ligand binding upregulates cyclic guanosine monophosphate (cGMP) levels. Cyclic GMP is a key second messenger which in turn drives the downstream activation of cGMP-dependent protein kinase type I (PKG). This leads to inhibition of the calcineurin-nuclear factor of activated T cells (NFAT) signaling pathway, which negatively regulates cardiac myocyte hypertrophy. This highlights the antihypertrophic effect of BNP[11] (Fig. 2).

Moreover, BNP plays a crucial role in regulating blood pressure levels by mediating diuresis, natriuresis, vasodilation, and by antagonizing the renin-angiotensin-aldosterone axis.

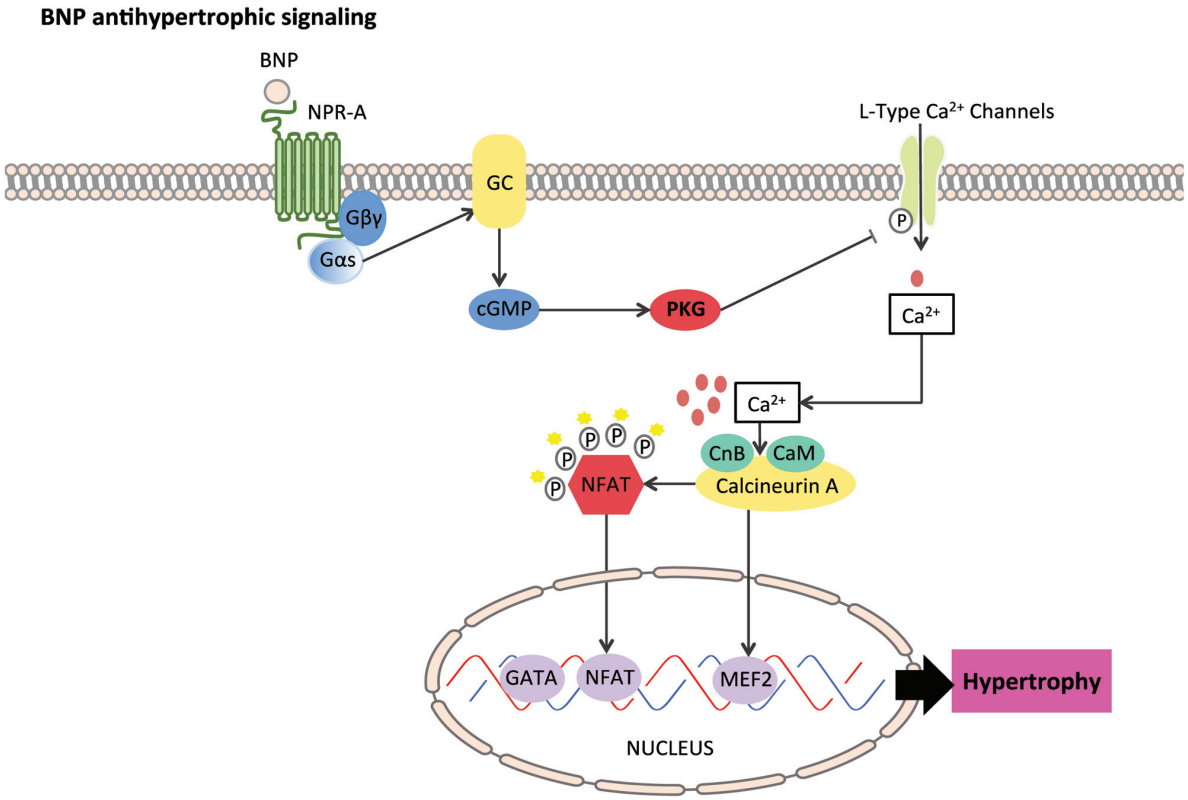
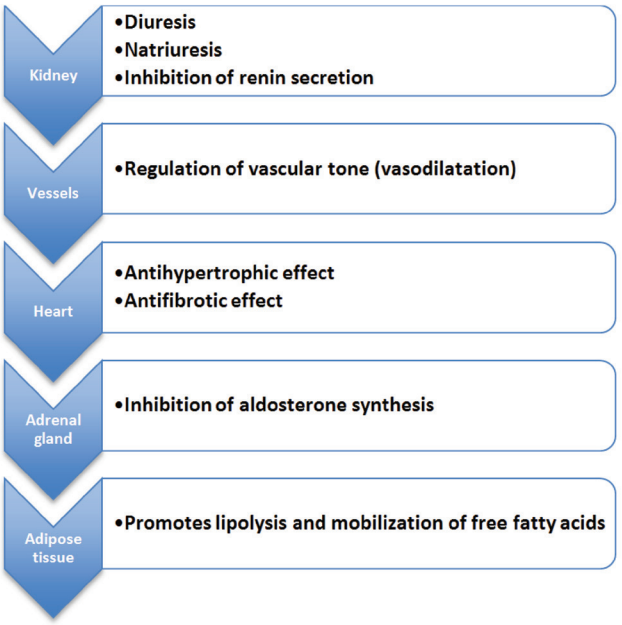


FIGURE 2. BNP antihypertrophic signaling pathway. BNP binds preferentially to the natriuretic peptide receptor-A (NPR-A) to exert its effect in the regulation of intravascular blood volume and vascular tone. NPR-A is linked to guanylyl cyclase (GC), which upon ligand binding upregulates cyclic guanosine monophosphate (cGMP levels). Cyclic GMP is a key second messenger which in turn drives the downstream activation of cGMP-dependent protein kinase type I (PKG). This leads to inhibition of the calcineurin-nuclear factor of activated T cells (NFAT) signaling pathway, which negatively regulates cardiac myocyte hypertrophy. BNP = brain natriuretic peptide; Ca2+ = calcium; CaM = calcium-modulated protein (calmodulin); CnB = calcineurin B subunit; Gαs = G protein α_s subunit; Gβγ = G protein βγ dimer; GATA = transcription factor binding to the DNA sequence "GATA"; MEF2 = myocyte enhancer factor-2 proteins (a family of transcription factors); P = phosphorus.

Increasing evidence supports the beneficial effects of BNP in attenuating or inhibiting the processes that contribute to cardiovascular remodeling, such as hypertrophy and fibrosis.[12] Additionally, it has been shown that in human adipocytes where NPR-A is expressed in significant amounts, upon BNP binding, cGMP becomes activated and promotes downstream lipolysis and mobilization of free fatty acids.[13,14] It is interesting to note that a recent study suggested that mice hearts lacking NPR-A, exhibited marked hypertrophy with interstitial fibrosis resembling that seen in human hypertensive heart disease. Thus, it can be assumed that BNP can also act as a paracrine antifibrotic factor to inhibit the proliferation of cardiac fibroblasts.[15] Furthermore, several studies have shown that natriuretic peptide levels are markedly increased in patients experiencing an acute coronary event. Even so, the role of BNP in the pathophysiology of acute MI remains to be elucidated. Particularly, a study by Kawakami and colleagues suggested that increased BNP plasma concentrations three days after an acute MI led to increased polymorphonuclear cell infiltration in the infarct area in BNP-transgenic mice compared to non-transgenic mice. This caused increased matrix metalloproteinase (MMP)-9 release by the stimulated neutrophils, and subsequently an increased risk of myocardial rupture in BNP-transgenic mice.[16] On the other hand, researchers have shown that administration of ANP at the time of reperfusion may exert cardioprotective effects against myocardial reperfusion injury in an acute coronary syndrome. This is attributed to ANP's ability to limit adhesion of polymorphonuclear neutrophils to hypoxic endothelial cells, thus decreasing their activation and their MMP-9 releasing properties[17] (Table 1).

A great bulk of data has been published regarding the metabolism and degradation of natriuretic peptides. From these reports, it is clear that C-type natriuretic peptide receptor, which binds ANP as well as BNP, is the most widely and abundantly expressed natriuretic peptide receptor, and is being involved in the systemic clearance of natriuretic peptides from the circulation, via a receptor-mediated internalization and degradation process. Notably, C-type natriuretic peptide receptor has only a short cytoplasmic domain with no guanylyl cyclase activity. Kidney glomeruli express high levels of C-type natriuretic peptide receptor and BNP highly depends on renal function for its clearance.[18] Specifically, it is well acknowledged that BNP and its cleavage equivalent amino terminal pro-B-type natriuretic peptide (NT-pro-BNP) are small molecular weight proteins, that are filtered relatively freely by the glomeruli and catabolized by tubular epithelial cells without any other processing, such as tubular secretion or active reabsorption, and return into the circulation. From a clinical point of view, it has been suggested that BNP concentrations are higher in heart failure patients and hypertensive subjects within the same range of glomerular filtration rate which strengthens the fact that these concentrations may reflect cardiac production rather than impaired clearance.[19,21]

TABLE 1. Physiological Actions of Brain Natriuretic Peptide (BNP)



In addition, to C-type natriuretic peptide receptor -mediated internalization, BNP is also metabolized by extracellular proteases.[19] Neprilysin (NEP), a neutral endopeptidase, is an extracellular protease that degrades endogenous vasoactive peptides, including natriuretic peptides. Interestingly, NEP cleaves human BNP at Met5-Val6 and Arg17-Ile18 residues, but not at the conserved Cys10-Phe11 bond, as occurs with ANP and CNP cleavage.[20] Inhibition of NEP increases the levels of natriuretic peptides, countering the neurohormonal overactivation that contributes to vasoconstriction, sodium retention and maladaptive remodeling.[19,21,22]

In recent years, McMurray et al suggested that valsartan/sacubitril (brand name Entresto, previously known as LCZ696, a mixture of the angiotensin receptor blocker valsartan and the neprilysin inhibitor sacubitril), a combination also described as an "angiotensin receptor-neprilysin inhibitor" (ARNi), is superior to angiotensin converting enzyme (ACE) inhibition alone in reducing the risk of death and hospitalization for heart failure patients with a reduced left ventricular ejection fraction. The same study provided strong evidence that an ARNi is superior to inhibition of the renin-angiotensin system alone (enalapril) in patients with chronic heart failure, which further supported the replacement of ACE inhibitors or angiotensin II receptor blockers (ARBs) with an ARNi in the management of chronic heart failure.[23]

Furthermore, in 2001, nesiritide, a recombinant human BNP, was approved by the US Food and Drug Administration for early relief of dyspnea in acute heart failure. However, several meta-analyses raised concerns regarding the renal

toxicity and mortality associated with the use of nesiritide in patients with acute heart failure, as well as the increased rates of hypotension observed in these patients.[24]

Summarizing, it is without question that both an angiotensin receptor - neprilysin inhibitor (ARNI), as well as nesiritide, a recombinant human BNP, have shown much potential in becoming the next big thing in the field of cardiovascular therapeutics. However, further research is needed to elucidate their clinical usefulness in patients with heart failure.

MEASUREMENT OF BNP LEVELS AND ACCURACY IN THE DIAGNOSIS OF HEART FAILURE

Heart failure is a complex chronic and progressive clinical syndrome where the heart cannot meet the increased metabolic demands of the body. It is a major cause of hospitalization and death and it affects approximately 2.3% of the population in the United States, particularly the elderly, representing thus a growing clinical and economic burden. Interestingly, hospital admissions due to heart failure have increased over the past years.[25] Heart failure is difficult to diagnose, and has always been a challenge for clinicians worldwide. There is increasing evidence that BNP is synthesized in the heart as a response to cardiac stress and neurohormonal activation. In particular, BNP levels are found to be elevated in patients suffering from congestive heart failure. In addition, the importance of BNP and its N-terminal peptide (NT-pro-BNP) in the evaluation of patients with acute symptoms, such as dyspnea in the emergency department, has been documented.[26] A recent diagnostic accuracy study, conducted by Zaphiriou et al in the UK, was designed to determine the diagnostic accuracy of BNP and NT-proBNP plasma measurements in 306 patients with suspected heart failure, referred by their general practitioners. This study confirmed the importance of BNP and its N-terminal peptide measurements as a rule-out test for the diagnosis of heart failure in the referred patients. Furthermore, a simple classification of the 12-lead ECG into normal or abnormal in the same study, added little value to ruling out heart failure in these circumstances.[27] Moreover, a meta-analysis denotes BNP to be an accurate biomarker of heart failure. Pooling data from the 20 studies included, researchers concluded that using a cut-off value of 15 pmol/L achieves high sensitivity.[28] BNP measurements below this cut-off excluded diagnosis in patients with suspected heart failure symptoms. It is critical to note that measurement of BNP plasma levels may play a significant role in the diagnosis of patients with diastolic heart failure, since the diagnostic accuracy of BNP was observed to be greater when the definition of disease used as the reference standard patients who were diagnosed as having heart failure with preserved left ventricular ejection fraction.[28] Apart from this, we should note that testing of BNP levels is easy and inexpensive to perform in clinical settings since researchers have developed a specific radioimmunoassay for human BNP with the use of a monoclonal antibody modeling it on a radioimmunoassay for ANP.[29] Observations from 1,586 patients presenting with dyspnea in the emergency setting in seven different departments across the USA, led to the use of 50 pg/mL as the cutoff BNP level, with low values shown to be highly sensitive at ruling out disease, whereas values above this level were found to be the strongest independent predictor of heart failure.[30]

Additionally, another study conducted by Dao et al recruited 250 patients presenting to the urgent-care area of the San Diego Veteran's Health Care System with acute dyspnea as the chief complaint.[31] BNP plasma concentrations were measured in all patients and emergency department physicians were blinded to the results. Consequently, two cardiologists (also blinded to BNP levels) reviewed all medical records of each patient retrospectively, and made independent initial assessments on whether patients' presenting dyspnea was secondary to heart failure. Researchers concluded that the mean plasma BNP level was higher in patients diagnosed with heart failure. Specifically, at a blood concentration of >80 pg/mL, BNP was considered as an accurate predictor of the presence of heart failure (95%), whereas measurements below this cut-off value had a high negative predictive value (98%). Thus, BNP was found to be a sensitive and specific test for the diagnosis of heart failure in the emergency setting.[31]

Importantly, it should be noted that the European guidelines for the diagnosis and treatment of acute and chronic heart failure propose a diagnostic algorithm or flow-chart for patients with suspected heart failure.[32] They recommend that BNP and NT-pro-BNP levels should be considered in order to exclude alternative causes of dyspnea (if the level is below the exclusion cut-off point: BNP <100 pg/mL, NT-proBNP <300 pg/mL) and to obtain prognostic information (Class IIa, Level C recommendation). For patients presenting in a non-acute setting, a lower exclusion natriuretic peptide cut-off point should be used to prevent a 'false-negative' diagnosis of heart failure (BNP <35 pg/m or NT-proBNP <125 pg/mL).[32]

BNP AS A PREDICTOR IN PATIENTS WITH CHRONIC HEART FAILURE

Brain natriuretic peptide (BNP), as well as its N-terminal peptide (NT-pro-BNP), are considered to be critical for the prognosis of heart failure progression and outcomes. Tsutamoto et al demonstrated for the first time that increased plasma BNP levels may be a critical prognostic predictor of heart failure progression in patients with left ventricular dysfunction.[33] Moreover, BNP measurement was found to be a better and independent predictor when used with hemodynamic parameters, such as pulmonary capillary wedge pressure

and left ventricular ejection fraction, to assess mortality in the same group of patients, compared to the plasma ANP level. As such, BNP and NT-pro-BNP neurohumoral factors are suggested as sensitive and promising biomarkers reflecting left ventricular dysfunction or damage, since BNP is ventricular in origin, with the latter being slightly superior.[33] In detail, scientists concluded that even the diagnostic and prognostic utility of NT-pro-BNP appears to be equivalent to BNP in the clinical setting, there are some biologic differences between them that render the N-terminal peptide a more accurate index of ventricular stress and therefore a better predictor of heart failure prognosis. It has been showed that NT-pro-BNP is not degraded in the blood circulation, and its structure remains stable even in the serum. Its half-life is approximately 1 to 2 hours, which is longer than the half-life of BNP. Conceivably, this leads to higher NT-pro BNP levels in the circulation and slower fluctuations than BNP.[34]

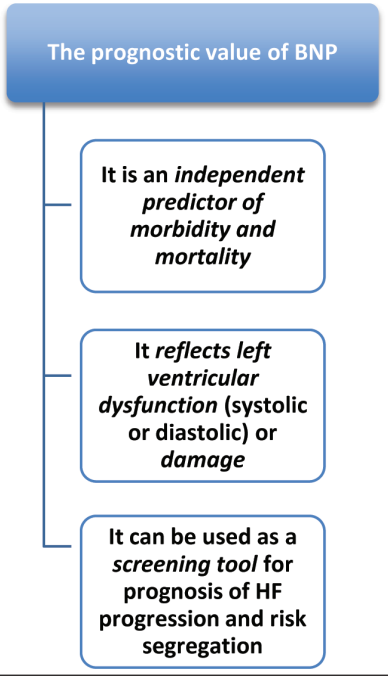
Additionally, an observational study monitored 325 patients presenting with dyspnea, as the major manifestation of heart failure, in the emergency department over a six-month follow-up period.[35] Subsequently, they concluded that higher BNP plasma levels at the emergency department were associated with cardiovascular events and progressively worse outcomes over the same period.[35] In recent years, Koglin et al designed a prospective cohort study to assess the relationship between plasma BNP values and risk stratification in patients diagnosed with congestive heart failure and who were considered to be at high risk for experiencing cardiac events, over a course of 398 days.[36] The results reinforced the usefulness of plasma BNP measurements as a screening tool for prognosis of heart failure progression and risk segregation, eliminating simultaneously the need for more invasive and expensive cardiac tests.[36] Similarly, another study was conducted in 452 patients with a left ventricular ejection fraction <35%, assessed by radionuclide ventriculography, to identify the role that BNP levels may play in predicting sudden cardiac death in this group of subjects, probably attributable to ventricular arrhythmias.[37] Following these patients for up to 3 years, they established BNP plasma levels as the only predictor of sudden cardiac death, with the 130 pg/mL being the cutoff value for this study.[37,38] Besides, Japanese scientists followed 290 consecutive patients with asymptomatic or minimally symptomatic left ventricular dysfunction for a mean period of 812 days in order to assess the plasma BNP level as an independent predictor of morbidity and mortality in this group of patients.[39] Blood samples were collected from all patients and plasma BNP levels as well as levels of other neurohumoral factors, such as ANP, norepinephrine, angiotensin II, and endothelin-1 were measured. Results indicated that plasma BNP concentration is a more useful biomarker for predicting morbidity and mortality than the other neurohumoral factors, and provides important information regarding hemodynamic parameters such as ejection fraction, left ventricular end-diastolic pressure, and left ventricular end-diastolic volume index, since the cardiac ventricles are the primary source of its blood level.[39] Finally, not only BNP is an important predictor of morbidity and mortality in patients with chronic symptomatic heart failure, but also predicts all-cause mortality in the general population and in those with no evidence of left ventricular systolic dysfunction[40] (Table 2).

SCREENING OF ASYMPTOMATIC SUBJECTS AT RISK FOR HEART FAILURE, SUCH AS THE ELDERLY, HYPERTENSIVE SUBJECTS, DIABETICS OR THOSE WITH ASYMPTOMATIC CORONARY ARTERY DISEASE

From a different perspective, it has been demonstrated that plasma BNP calculations may also reflect the risk for cardiovascular events, not only in heart failure patients with severe dyspnea, but also in asymptomatic subjects at risk for cardiac pump failure, such as the elderly, diabetics, patients with hypertensive heart disease, as well as those with asymptomatic coronary artery disease.

Specifically, a randomized clinical trial comprising 1,257 patients was designed to examine the cost-effectiveness of using plasma BNP as a screening test for assessing left ventricular systolic dysfunction in the general population, to further reduce the need for echocardiograms.[41] Consequently, BNP

TABLE 2. Prognostic Value of BNP



BNP = brain natriuretic peptide; HF = heart failure

was found to have high sensitivity in detecting left ventricular systolic dysfunction in low and high-risk patients with ischemic heart disease.[42] Indeed, appropriate treatment of heart failure led to improved survival and thus, an early diagnosis and proper decision making are therefore beneficial. Japanese researchers conducted a multiple regression analysis to evaluate systolic and diastolic cardiac function in seemingly healthy asymptomatic subjects by measuring BNP levels. In order to come to a conclusion regarding their assumption, they compared BNP levels with other cardiac indices obtained from chest radiographs, electrocardiograms and echocardiograms. Derived data from a pool of 294 asymptomatic patients showed a significant correlation between echocardiographic parameters and increased BNP plasma concentrations and both left ventricular systolic and diastolic functions, highlighting thus the effectiveness of BNP as a screening tool to reflect both the systolic and diastolic function.[42] Furthermore, left ventricular wall thickness, blood pressure measurements, and serum creatinine levels were found to correlate with raised BNP concentrations.[42] Cardiologists have also reported that by performing a simple radioimmunoassay for BNP, clinicians can rapidly and reliably detect the presence of significant diastolic filling abnormalities on echocardiography. Interestingly, low BNP concentrations along with normal echocardiogram performance may be able to rule out diastolic dysfunction, whereas raised BNP values in heart failure patients with preserved ejection fraction, especially the elderly, are found to be associated with diastolic dysfunction on Doppler studies.[43] More specifically, it has been shown that screening for asymptomatic left ventricular dysfunction is of particular importance as its prevalence alone ranges from 0.9 to 12.9%, depending on standard risk.[44] Indeed, in the general population, patients with increased plasma BNP levels exhibit significant morbidity and mortality and thus, accurate screening of subclinical disease is necessary.[45-48]

FUTURE DIRECTIONS

Surely, BNP may be a promising biochemical marker of effective blood pressure management, since multiple studies have suggested that its measurement probably could be a guide of hypertension management and target organ protection. However, further cutting-edge research is needed to elucidate the role of BNP plasma concentrations in the assessment of cardiovascular risk and mortality.

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