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Review article
Pregledni članak

DETERMINATION OF B-TYPE NATRIURETIC PEPTIDES: CLINICAL AND ANALYTICAL QUALITY

ODREĐIVANJE B-TIPA NATRIURETSKIH PEPTIDA: KLINIČKI I ANALITIČKI KVALITET

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Summary: B-type natriuretic peptide (BNP), a neurohormone synthesized in the cardiac ventricles, is released as preproBNP and then enzymatically cleaved to the N-terminal-proBNP (NT-proBNP) and BNP upon ventricular myocite stretch. This hormone has a role in the body's defense against hypertension and plasma volume expansion. Measurements of circulating B-type natriuretic peptides have been shown to be of diagnostic value in patients with heart failure. BNP levels correlate with the severity of heart failure, as well as with prognosis. Better control of the preanalytical and analytical sources of variations will undoubtedly lead to improvement in B-type natriuretic peptides measurements. A number of preanalytical and analytical factors including specimen type and stability, assay imprecision, and standardization are reviewed here. Further research is required to better define the performance characteristics necessary for assays bearing the designation natriuretic peptides. These characteristics include developing guidelines for the total analytical error from a careful review of the intraindividual biological variability of the analyte under conditions that will be encountered in clinical practice, then validating these guidelines in the clinical setting, and completing the standardization efforts.

Keywords: brain natriuretic peptide, NT-proBNP natriuretic peptide, ventricular dysfunction, heart failure, determination of B-type natriuretic peptides

Kratak sadržaj: Neurohormon koji se sintetiše u srčanim komorama, B-tip natriuretskog peptida (BNP), usled rastezanja zidova komora oslobađa se kao preproBNP i enzimski se cepa na N-terminalni-proBNP (NT-proBNP) i BNP. Ovi hormoni štite organizam od povećanja pritiska i povećanja volumena tečnosti. Merenje vrednosti B-tipa natriuretskih peptida je značajno za dijagnostikovanje pacijenata sa srčanom insuficijencijom. Vrednosti cirkulišućeg B-tipa natriuretskih peptida kod ovih bolesnika koreliraju sa težinom bolesti i prognozom bolesti. Bolja kontrola preanalitičkih i analitičkih izvora varijacija sigurno vodi poboljšanju određivanja B-tipa natriuretskih peptida. U ovom radu je opisan veliki broj preanalitičkih i analitičkih faktora, kao što su vrsta uzorka, stabilnost, nepreciznost određivanja i standardizacija, važnih za određivanje B-tipa natriuretskih peptida. Neophodna su dodatna ispitivanja vezana za definisanje karakteristika izvođenja testova za određivanje B-tipa natriuretskih peptida. Ove karakteristike uključuju preporuke vezane za ukupnu analitičku grešku nastalu zbog intraindividualne biološke varijacije B-tipa natriuretskog peptida, koja mora da se uzme u obzir pri donošenju kliničkih odluka. Neophodno je da se izvrši validacija ovih preporuka u specifičnim kliničkim situacijama i završe aktivnosti vezane za standardizaciju određivanja.

Ključne reči: B-tip natriuretski peptid, NT-proBNP natriuretski peptid, disfunkcija komore, srčana insuficijencija, određivanje B-tipa natriuretskih peptida

Introduction

The identification of natriuretic peptides led to an explosion of basic and clinical investigations with the aim to clarify their physiology, pathophysiologic role in heart failure, and clinical usefulness (1). The natriuretic peptide family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and urodilatin. ANP and BNP are cardiac hormones with similar peptide chain as well as degradation pathways, while other natriuretic peptides, such as CNP and urodilatin, are not produced and secreted by cardiac tissue but by other

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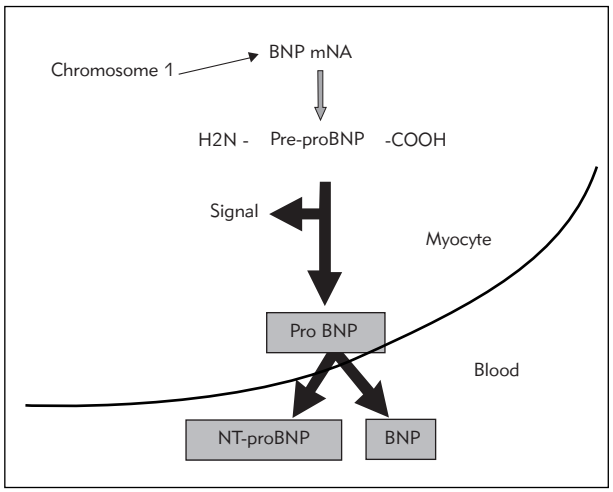

Figure 1 Synthesis and secretion of B-type natriuretic peptide (BNP) (1).

tissues (2). These peptides are characterized by a common 17 amino acid ring structure with a disulfide bond between two cysteine residues. This ring structure shows high homology between different natriuretic peptides, and it is essential for receptor bindings and the biological activity of natriuretic peptides.

Regulation of BNP synthesis and excretion occur mainly at the level of gene expression. The secretion and mRNA turnover of BNP are faster than those of ANP. The nucleic acid sequence of the BNP gene contains the destabilizing sequence »TATTTAT« (T=timin, A=adenin) which suggests that turnover of BNP mRNA is high, and that BNP is mainly synthesized in bursts (3–5). During secretion, a 108 amino acid pro-BNP is separated into a 32 amino acid carboxy-terminal biologically active portion (BNP) and a 76 amino acid amino terminal part (NT-proBNP-1-76) without biological activity (*Figure 1*).

Effects of natriuretic peptides are realized through mechanisms which are characteristic of all hormones. Natriuretic peptide is the first messenger which is bound to highly specific receptors and it realizes its intracellular effects by the second messenger. Three types of these receptors are verified: natriuretic receptor type A, natriuretic receptor type B, and natriuretic receptor type C. Although A and B types of natriuretic receptors have many similarities, intracellular parts are not completely homologous, which points to two different regulatory systems. Natriuretic receptor type C serves as clearance receptor. These highly specific receptors have been identified at target sites and kidneys. Most cardiovascular and renal effects of ANP and BNP result from cyclic-guanyl-monophosphate formation which acts as a second messenger responsible for the cellular physiological responses to natriuretic stimulation.

Plasma BNP natriuretic hormone has weaker affinity for clearance receptors and enzyme endopeptidase than ANP. The plasma half-life of circulating BNP (20 min) is longer than the half-life of ANP (3 min). The biological half-life of N-terminal fragment NT-pro-BNP is 120 min. Natriuretic peptides are metabolized through the type C receptor. Removal of these peptides from circulation is conducted by enzyme degradation and glomerular filtration.

Effects of natriuretic peptides

Natriuretic peptides play a key role in blood pressure and volume homeostasis. Increased secretion of natriuretic peptides reduces blood pressure and plasma volume through coordinate action in the brain, adrenal gland, kidney, and vasculature (1). Natriuretic peptides increase the hematocrit values, most probably due to drop of plasma volume because of shifting the liquid on the capillary level from intravascular to interstitial space (6). This effect is realized by the increased permeability of vascular endothel and increased hydrostatic pressure in the capillaries. Drawing liquid from blood vessels into extravascular space is not the only mechanism for the reduction of heart overload. Natriuretic peptides oppose the vasodilatation of veins which decreases the influx into right heart and decreases minute volume. On the level of regional blood flows, natriuretic peptides increase renal plasma flow and flow through the hepatic circulation. Natriuretic peptides influence smooth muscle cells of the blood vessels. Vasodilatory effect, i.e. vasorelaxation effect, is more obvious in arteries than in veins.

Natriuretic peptides reduce sympathetic tone in the peripheral vasculature. This reduction is probably caused by the dampening of baroreceptors, suppression of the release of catecholamines from autonomic nerve endings, and especially by suppression of sympathetic outflow from the central nervous system (7, 8). Natriuretic peptides lower the activation threshold of vogues activity which leads to suppression of the reflex tachycardia and vasoconstriction that accompany the reduction in preload, ensuring a sustained descrease in mean arterial pressure (9). Natriuretic peptides have antimitogenic effect on smooth muscle cells of the blood vessels. Renal actions of ANP and BNP directly influence the tubules and inhibit the tubular ion reabsorption. Another mechanism of natriuretic peptide effects on electrolyte excretion is associated with redistribution of circulation within the kidney and with changes in renal circulation. When it comes to the effect on renal circulation, as shown by the hemodynamic results of many studies, natriuretic peptides increase renal blood flow and increase glomerular filtration. The most important effect that natriuretic peptides have is on the renin-angiotensin-aldosterone system. They are physiological antagonists of the renin-angiotensin-aldosterone system. Under the influence of natriuretic peptides large quantities of sodium reach distal tubules and through masculae dense inhibit renin secretion (10). Natriuretic peptides

inhibit biosynthesis and secretion of aldosterone by directly influencing the adrenal *zona glomerulosa*. These peptides realize their effects by opposing the reabsorbing effect of aldosteron on sodium ions. Most articles show that natriuretic peptides antagonize the vasoconstriction effect of angiotensin II.

The actions of natriuretic peptides in the brain reinforce those in the periphery. Therefore, the peripheral natriuretic effects are amplified by the central inhibition of salt appetite and water intake, which complements the renal diuretic effects of the peptide (11, 12). ANP inhibits secretion of vasopressin and, in some studies, corticotrophin through effects on the brain and pituitary gland (13).

The role of natriuretic peptides in heart failure

Heart failure (HF) can be the result of any condition that decreases the ability of the heart to pump blood in order to meet the body's need for oxygen. The cause is usually decreased myocardial contractility due to heart damage (AMI, toxins, infections, stress, etc.). The heart can improve its decreased function by a long range of adaptive mechanisms (Frank-Straling mechanism, myocardial hyperthrophy with or without dilatation, activation of the neurohormonal system) which improve arterial blood pressure and perfusion of vital organs. Low-output HF leads to arterial underfilling which sets into motion a series of baroreceptor-mediated events with the goal to restore arterial circulatory integrity (14). Neurohumoral activation of the renin-angiotensin-aldosteron system and activation of the sympathetic nervous system play, in the beginning, a compensatory role in order to improve the weakened functions of the heart. Increased level of catecholamines by positive inotropic effect increases contractility of the heart muscle, while by positive chronotropic effect, that is, increased heart rate, it increases the decreased minute volume. The resulting vasoconstriction increases filling of the heart and stimulates thirst. Renal salt and water retention occur with a delay of several days. However, this compensatory effect soon becomes deleterious for the weakened heart muscle. Function of the heart is weakened more and more, and neurohumoral activation gains opposite effect. Beside that, it is well known that catecholamines have toxic effect on the myocardium, and that is the initial link in further progress of myocardial fibrosis. Catecholamines can be responsible for the hyperthrophy process which leads to the remodeling of the chamber. Higher level of catecholamines increases the heart rate and at the same time causes coronary vasoconstriction. The coronary flow is then decreased as well as the myocardial contractility. At the cell level it influences growth factors and the apoptosis.

Natriuretic peptides have an important role in maintaining the compensated state of HF and delaying progression of the disease. Cardiac hyperthrophy, which usually accompanies heart failure, stimulates the production of ANP and BNP, and their release is further stimulated by stretching of the failing atria and ventricular myocardium (15, 16). The mechanism of an increased secretion of natriuretic peptides can be explained by chronic central hypervolemia with increased ventricular filling pressure (17). Natriuretic peptides protect the body from excessive quantities of salt and water, inhibit production and effects of vasoconstriction peptides and influence the vascular relaxation. They inhibit the release of ACTH and the sympathicus effect. On the peripheral level, they increase the filtration rate, diuresis, natriuresis, decrease the systemic vascular resistance and plasma volume in order to protect the heart from overload. In the progression of the disease vasoconstriction effects and retaining of salt and water take prevalence and natriuretic peptides can work no longer against the renin-angiotensin-aldosteron system and the sympathetic nervous system. This condition leads to appearance of the clinical signs and symptoms of HF. ANP and BNP plasma concentrations correlate with the level of disease according to the New York Heart Association (NYHA) functional class (18). In further progression of the disease glomerular filtration and urine flow decrease, and sodium absorption increases in the proximal tubules with the decreased sodium flow in the collecting ductus where natriuretic peptides act. In patients with HF, ANP and BNP are synthesized in ventricles and atria, but their response is inadequate.

Clinical applications

Heart failure

Numerous studies have consistently found that these peptides are elevated in patients with HF and

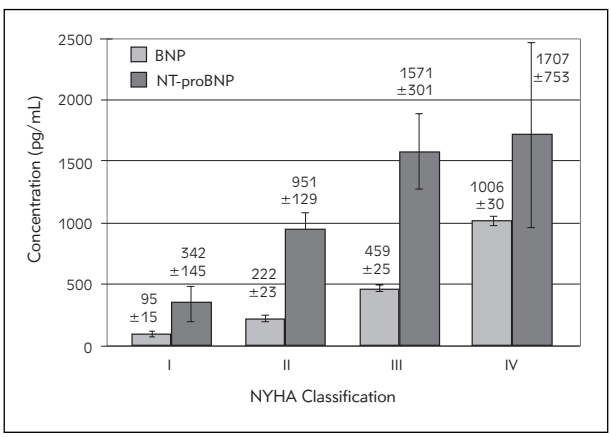

Figure 2 Relationship between B-type natriuretic peptide (BNP) and N-terminal (NT)-proBNP and the New York Heart Association (NYHA) functional classification. Data from Roche Diagnostics (20) and Biosite Inc (21).

the values were found to be related to disease severity as assessed by the NYHA functional class (*Figure 2*), left ventricular systolic ejection fraction, and left ventricular diastolic function (18–22). BNP is the emergency hormone that responds immediately to ventricular overload. Release of BNP appears to be directly proportional to the degree of ventricular volume expansion and pressure overload (23). High levels of these heart hormones point to the presence of a high risk for the development of fatal cardiovascular complications, including death (24, 25). Determination of these peptides is also clinically significant as an exclusion test in HF diagnosis, considering their highly negative predictive importance.

Acute setting

Several studies confirmed that determining BNP and NT-proBNP concentrations can help clinicians in the differential diagnosis of acute dyspnea. In a study of 250 patients presenting to emergency department with dyspnea, a BNP of >80 pg/mL was 98% sensitive and 92% specific for an ultimate diagnosis of congestive heart failure (CHF) (26). In another emergency room study of 321 patients with severe dyspnea, a BNP of >94 pg/mL was 86% sensitive and 98% specific of an eventual clinical diagnosis of CHF (27). Using the cutoff of 100 pg/mL, the sensitivity and the specificity of BNP level were 90% and 76%, respectively; the cutoff of 50 pg/mL was assessed with the sensitivity of 97% and the specificity of 62% in a multicentric study of 1586 patients presenting with acute dyspnea not obviously caused by CHF (23). In a similar primary practice cohort, the use of NT-proBNP improved the accuracy of HF diagnosis by 21%, whereas repeated clinical evaluation without prior knowledge of NT-proBNP improved diagnosis by only 8% (28). Low BNP or NT-proBNP concentrations (<100 ng/mL or <300 ng/mL, respectively) during the initial approach to patients with suspected HF can confidently exclude heart failure diagnosis. A positive result should lead to investigations in order to detect the cardiac functional and structural abnormalities or other conditions associated with raised BNP concentrations (mild to severe chronic obstructive pulmonary disease, renal failure, myocardial ischemia, atrial fibrillation) (1).

Combination of a strategy based on BNP determination and clinical assessment is the ideal approach to optimize early diagnosis and intervention (29, 30). The presence of markedly raised levels of BNP and NT-proBNP may help to target those for subsequent detailed assessment of underlying cardiac dysfunction using more specialized procedures (e.g. echocardiography, radionuclide ventriculography, exercise testing, etc.).

The prognostic value of BNP and NT-proBNP

These markers are beneficial because of their prognostic value. Several large-scale studies have convincingly shown that BNP and NT-proBNP provide strong prognostic information for an unfavourable outcome (death, cardiovascular death, readmission or cardiac events) in patients with heart failure or asymptomatic left ventricular dysfunction (31). Both BNP and NT-proBNP are independent predictors of high left ventricular end-diastolic pressure and are more useful than ANP or other neurohormones for assessing mortality in patients with chronic HF (32). In moderate to severe HF patients (left ventricular ejection fraction <25%), both BNP and NT-proBNP were independently related to death within four years, and survivors showed increasing left ventricular ejection fractions, in parallel with the decreasing NT-proBNP values (33). Patients with the highest NT-proBNP levels had a very poor short-term prognosis (34).

It has been verified in studies that the BNP is the best predictor of 30-day mortality in patients with acute coronary syndromes (35). Other authors have shown that the NT-proBNP level above the median value predicts four-year mortality in acute coronary syndromes (36).

Natriuretic peptides have also been used to estimate prognosis in other cardiovascular conditions (valve disease, acute pulmonary embolism).

Coronary artery disease (CAD)

It is well known from experimental studies and clinical investigations in humans that natriuretic peptides are released from ischemic cardiomyocytes, and that hypoxia per se stimulates the release of these peptides from the myocardium (37). Cross-sectional data from two small-scale studies have suggested that circulating BNP and NT-proBNP, but not ANP levels, are higher in patients with unstable angina than in patients with stable coronary artery disease (38, 39). A significant net release of BNP occurred during reperfusion after cardioplegic cardiac arrest in patients undergoing uneventful coronary artery bypass grafting, and plasma BNP closely correlated with myocardial lactate production as well (40). It has also been demonstrated that BNP even rises after temporary myocardial ischemia induced by balloon inflation during coronary intervention.

Acute coronary syndromes (ACS)

Elevated BNP and proBNP concentrations do not necessarily reflect HF, but may also result from cardiac ischemia. Recent studies have shown that plasma concentrations of BNP and proBNP are elevated in patients presenting with unstable coronary

syndrome or myocardial infarction. Natriuretic peptides greatly increased in AMI patients during the acute phase, perhaps as a compensatory reaction (41). This increase is probably due to several factors, including alterations in hemodynamics, ischemia, increased synthesis, especially in the periinfarction zone, or release from the necrotic myocardium (37). Goetze et al. suggest that ventricular BNP gene expression is up-regulated by myocardial hypoxia, resulting in augmented plasma concentrations of BNP and pro-BNP (42). Sustained elevation of plasma BNP up to 90 days after AMI was associated with progressive ventricular remodeling in large transmural infarction (43). In various studies in patients with ACS different cutoff values have been applied, but to date no clearly defined cutoff has been established. Furthermore, both markers are highly predictive for an adverse outcome independently of other biomarkers, especially troponins and C-reactive protein (CRP). In the FRISC-II trial serial NT-proBNP analyses were evaluated during the acute and chronic phase of ACS, and disclosed that the predictive value of NT-proBNP value measured three and six months after the index event is a better predictor for two-year mortality than early NT-proBNP determination at admission or at 48 h after the acute event (44). Plasma BNP and proBNP concentrations provide prognostic information and are prognostic markers of morbidity and death in patients presenting with acute coronary syndrome.

BNP for monitoring heart failure treatment

Data from studies are promising and suggest that BNP and NT-proBNP might be clinically useful for determining the optimal treatment for patients with HF and for monitoring treatment effects (22, 45). However, most studies report mean natriuretic peptide changes in parallel with different criteria for the improvement of HF without showing the individual course of a patient. Criteria, such as cardiothoracic ratio and left ventricular end-diastolic diameters or increases in maximal exercise capacity during the bicycle exercise stress test and improvement in NYHA class, were associated with decreases in BNP concentrations during follow-up of the congestive HF patients (46–47). A study of 69 patients with symptomatic HF found significantly fewer total cardiovascular events (deaths, hospital admissions or episodes of HF decompensation) in the group randomized to NT-proBNP guided therapy compared to a similar group of patients whose therapy was guided by the commonly used clinical variables (48). Furthermore, patients with mild forms of HF may benefit from disease monitoring with natriuretic peptides. In patients with ischemic cardiomyopathy, elevation of BNP without marked activation of norepinephrine identified a group who had the best response to beta-blockade with carvedilol (49). However, only moderate but equal diagnostic performances of changes in BNP and NT-proBNP to differentiate between HF patients with and without improvement in the clinical HF status were found during a 3-month follow-up, and, therefore, natriuretic peptides cannot replace the already established methods (50). Early clinical experience in applying these findings to individual patients, however, suggests that the intraindividual biologic variation of BNP may make interpretation of serial measurements difficult (51). Therefore, only marked BNP or NT-proBNP changes during follow-up are related to changes in the clinical HF status.

Natriuretic peptides are increased in all diseases affecting the cardiac or renal function and fluid balance. However, BNP rises as renal function declines, so reduction of BNP based on diuretics may result in worsening renal function and subsequent increases in BNP concentrations (1).

The causes of variability in NP measurement

Obtaining of reliable results during NP measurements requires good recognition of all preanalytical and analytical variables, which may influence the NP values. The preanalytical ones include: physiological factors (age and gender, lifestyle, obesity, drug use, and some other conditions) and sample collection (blood sampling conditions, sample type, time and temperature of sample preservation). Analytical variables include: measurement methods, standardization of assays, limit of detection, linearity, imprecision of methods, reference limit.

Preanalytical issues

Gender and age: uniformly across community cohorts women have higher BNP values than men of the same age strata. Estrogens stimulate the natriuretic peptide production in females (52). Research showed that secretions of BNP and NT-proBNP are affected by age. Blood concentrations of these peptides increase with age, presumably as a result of left ventricular stiffness and progressive deterioration of renal function.

Biological variability: a lack of understanding of the physiological and biological variability of BNP and NT-proBNP in HF patients may lead clinicians to misinterpret changing (increasing or decreasing) BNP and NT-proBNP concentrations in the context of establishing the success of failure therapy. It has been reported in the literature that both BNP and NT-proBNP exhibit an intraindividual biological variability of 30–50%. Therefore, only marked BNP or NT-proBNP changes during follow-up are related to changes in the clinical HF status.

Obesity: obesity has also been shown to have an impact on BNP measurements; there is an inverse

relationship between BMI increase and BNP decreases. The observation of lower concentrations of BNP in obese people remains unexplained.

Pathological state: patients with severe lung disease, hypertension, and diabetes may have higher BNP and NT-proBNP concentrations than their age-matched controls. Plasma values of these peptides are also increased in patients suffering from chronic renal failure or liver cirrhosis with ascites.

Drugs: blood samples should ideally be drawn before the start of therapy in HF patients. Drugs used in HF treatment can modify circulation concentrations of BNP. Beta-blockade may have a variable effect on circulating BNP concentrations (53). Diuretics and vasodilators reduce BNP concentrations rapidly (together with falling intracardiac filling pressures); angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, and spironolactone also lead to decreases in BNP concentrations.

Other conditions: habits, such as sodium intake, may influence the NP levels. Increased plasma levels have also been reported in the last trimester of pregnancy and in the immediate puerperium (54).

Blood sampling conditions: blood should be drawn only after the standardized period of rest because NPs may increase with exercise. In most studies on NPs in HF duration of rest was usually 10 minutes. In all follow-up investigations, the position of the patient should always be the same, either supine or sitting. To eliminate the effects of diurnal fluctuations of NPs concentrations, blood should be drawn at the same time of the day during the follow-up. However, in healthy subjects, no day-to-day fluctuations or circadian rhythm have been reported for BNP when determined every third hour over 24 hours (55).

Sample type: for BNP assays, EDTA anticoagulated whole blood or plasma appears to be the only acceptable specimen choice. Only one system, the Biosite Triage meter, allows for the direct measurement of whole blood (EDTA) BNP. At present, it appears that for the Elecsys NT-proBNP assay, serum is the matrix of choice. However, for NT-proBNP, heparin plasma and EDTA plasma (reads 10% lower) appear acceptable.

Time and temperature of sample preservation: specimens for BNP measurement may be stored at ambient temperature for 24 h or at 30 °C for 12 h; EDTA plasma is stable at –20 °C for one month or, with an addition of the protease inhibitor aprotinin, longer (56). BNP is reportedly unstable when collected in glass tubes because of the activation of kallikreins of the extrinsic clotting pathways, but this phenomenon may be dependent on the specificities of antibodies used in the measurement method (57). Neuroendopeptidases (NEPs) are not likely involved with *in vitro* degradation because BNP degradation continues after the deactivation of NEPs by EDTA chelation (58).

NT-proBNP appears to be relatively stable during the sample storage. Measured concentrations in serum, heparinized plasma, and EDTA plasma are stable in samples stored at room temperature or 4 °C for at least 72 h (59). Samples are also stable for at least one year when stored at –80 °C, and five freeze-thaw cycles had no effect on analyte concentration (60).

Analytical issues

Measurement methods: at present, there are several commercially available assays for BNP and NT-proBNP for routine clinical practice. BNP can be assayed on Access 2 BNP (Biosite, San Diego, CA), or by a rapid fluorescence immunoassay Triage meter BNP assay (Biosite), an enzyme immunoassay (AxSYM BNP, Abbott Diagnostics, IL), or a chemiluminescent immunoassay (ADVIA Centaur BNP, Bayer Diagnostics, NY) and NT-proBNP can be measured by an electrochemiluminescent assay (E170, Roche Diagnostics, IN).

Standardization of assays: currently available commercial assays for BNP and NT-proBNP are not standardized, i.e., they have not been calibrated against common standards. These assays have different characteristics, and their relative performance has only begun to be evaluated. Possible reasons for the nonharmonization of methods are differences in the peptide calibrators used and variation in assay antibody reactivity to the analyte forms that may be present in blood, which would lead to varying total immunoreactivity among assays (61). Therefore, reference intervals and decision limits derived from clinical studies are only valid for the particular assay used and must not be extrapolated to other assays.

Interferences: assays for BNP and NT-proBNP may differ in their susceptibility to analytical interferences. Interferences from heterophilic antibodies, such as rheumatoid factor, or from human anti-animal antibodies (HAAAs) may lead to false test results (62). Icteric and hemolyzed samples might also be a problem in certain immunoassays with fluorometric detection of the signal.

The limit of detection: for each automated natriuretic peptide method the limit of detection is lower than the manufacturers' claimed values. The limit of detection for each method is lower than 9 ng/L (63).

Linearity: the target value for each linearity sample was calculated based on the samples with the lowest and the highest concentrations within the analytical measurement range for each method. All methods had a maximum average deviation from the target recovery of less than 10% (63).

Imprecision of methods: imprecision studies were performed using individual manufacturers' quality control material. All methods demonstrated total imprecision of less than 10% (63).

Reference limit: the upper 97.5 % reference limit for all methods fell below the manufacturers' clinical decision thresholds. The AxSYM and E170 had upper limits that were only slightly lower than the manufacturers' diagnostic cutoff values. The AxSYM had upper reference limit of 79 ng/L with the diagnostic cutoff of 100 ng/L, and the E170 had an upper reference limit of 114 ng/L with the diagnostic cutoff of 125 ng/L. The Access 2 BNP and ADVIA Centaur BNP, however, had upper reference limits of 42 ng/L and 37 ng/L, respectively, that were much lower than the diagnostic cutoff of 100 ng/L for each assay (63).

Conclusion

Plasma brain natriuretic peptide (BNP) and NT-proBNP are considerably increased as a result of myocardial stress caused by various cardiovascular diseases. In the clinical practice, BNP testing is best used as a »rule out« test for the suspected cases of new HF in breathless patients presenting to either the outpatient or emergency care settings. Both markers can be measured using fully automated commercially available assays. The assays have not been standardized so far and the application of various calibration materials may contribute to different results. Although precise cutoff values of BNP and NT-proBNP have not been established as yet, heart failure appears to be very unlikely below the plasma concentrations of 100 pg/mL and 300 pg/mL, respectively. The high biological variation of natriuretic peptides must be considered when interpreting serial BNP and NT-proBNP results. In heart failure patients BNP is probably useful as a marker of prognosis. It can help to identify those at highest risk of mortality and recurrent hospitalization. Recent studies demonstrated the usefulness of these markers in guiding and monitoring heart failure treatment.

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