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CLINICAL CHEMISTRY
NO. CC 12-3 (CC-401)

Volume 52
Number 3
ISSN 1056-599x
May 2012

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- explain the basic features of heart failure and its relationship to natriuretic peptide release.
- describe analytic differences between BNP and NT-ProBNP.
- describe clinical advantages of one natriuretic peptide over another.
- explain the differences among the different B-type natriuretic peptides.

HISTORY

Multiple laboratory instrument manufacturers were being considered to provide laboratory analyzers for a consolidated laboratory intended to support multiple hospitals within a hospital system. The systemwide clinical pathologist reviewed all instrument chemistry test menus to identify any significant assay-related, analytic, or clinical differences among the instrument platforms of the different manufacturers. Most assays were comparable across manufacturers, with only slight variation in methods or reference ranges. The pathologist identified 2 analytes that differed when compared with the existing instrumentation and among the various proposals submitted, troponin (troponin T vs troponin I) and natriuretic peptide (NP) (brain natriuretic peptide [BNP] vs N-terminal proBNP [NT-ProBNP]) assays. Because cardiologists were the physician group that most frequently used these assays, the pathologist consulted the chairman, medical director, and associate medical director of the cardiology departments of the hospitals in the system about the variation in troponins and NPs. The cardiologists agreed that any troponin analyzer was acceptable, but had varying opinions about analyzers for BNP vs NT-ProBNP. Because of the controversy within the cardiology leadership, the chairman of the cardiology department asked the clinical pathologist to review BNP vs NT-ProBNP and to present a succinct discussion of the issues relevant to the hospital system's choice of manufacturers.

BNP VS NT-ProBNP IN CLINICAL USE

There are 3 NPs: A, B, and C. All 3 share a 17–amino acid ring structure and commonality of physiologic effect: they mediate a cardiovascular-renal response to volume overload through natriuresis, diuresis, vasorelaxation, and sympatho-inhibition. A-type NP storage granules were identified in the 1950s, and their related substance, atrial NP, was isolated in 1984. In 1988, a circulating peptide with similar biochemical structure and function was identified and isolated from porcine brain and named *brain natriuretic peptide* (BNP). It was shown that most BNP in circulation originates in the heart. Shortly after these discoveries, the same research group identified C-type NP.

C-type natriuretic peptide is derived primarily from endothelial cells. A-NP is primarily synthesized and stored in granules in the atria, and B-NP is synthesized and released from both atria and ventricles. (B-NP refers to the generic group of all B-type NPs, whereas BNP refers to the specific active fragment of ProBNP, discussed below.) Per weight, there appears to be more B-NP in the atria than in the ventricles; however, the greater mass of the ventricles suggests that more B-NP messenger RNA is present, raising the probability that most circulating B-NP is ventricular in origin.[1] Pathophysiologically, all the NPs are linked by their actions in response to heart failure.

Heart failure is the clinical term for a decline in the heart's ability to pump blood. It can occur over a long period with an insidious onset, such

as in genetic cardiomyopathy, or it can develop rapidly, secondary to an acute event such as myocardial infarction. As the heart's ability to pump blood decreases, cardiac output drops; as cardiac output declines, compensatory mechanisms maintain output. These mechanisms include the sympathetic nervous and the renin-angiotensin-aldosterone systems, which cause salt/water retention, systemic vasoconstriction, and increased myocardial contractility.[2,3] These compensatory mechanisms are not long-term solutions, and their effects contribute to the morbidity and mortality associated with heart failure.

The NPs counteract the compensatory mechanisms. NPs are synthesized and released in response to increased atrial and ventricular wall stretch secondary to increased intravascular volume caused by sodium retention. The NPs block tubular sodium reabsorption, increase glomerular filtration rate (GFR), and inhibit the sympathomimetic nervous system. As a result, sodium and water excretion and systemic vasodilation both increase.[1] These effects ultimately lead to increased natriuresis and diuresis.

BNP synthesis and release is triggered by the increased myocardial wall tension that occurs with increased circulating volume. Increased wall stretch[4] induces translation of the BNP gene, located on chromosome 1, to produce PreProBNP (134aa + 28a), a signal peptide. The signal peptide is rapidly cleaved to form ProBNP. ProBNP is then cleaved by 2 prohormone convertases, furin and corin, followed by rapid posttranslational modification to form, during its release, BNP (32aa), a physiologically active fragment, and N-terminal ProBNP (NT-ProBNP) (76aa), an inactive fragment. Historically, it was believed that the release of BNP and NT-ProBNP was equimolar and limited to only those molecules. We now know that a small fraction of ProBNP and multiple fragments of all 3 molecules over a range of molecular weights are released.5,6 To what degree ProBNP and the BNP fragments (not NT-ProBNP) exert a physiologic effect is debated. BNP is the only B-NP that is known to have a significant physiologic effect.

The diagnosis of heart failure can be difficult, as it is largely based on a clinical assessment to determine if the patient is in a volume-overload status that is cardiac in origin.2 Heart failure symptoms are associated with volume overload and include dyspnea, orthopnea, and paroxysmal nocturnal dyspnea. A multitude of other signs, symptoms, and findings, as well as a recommended algorithm for diagnosis using these findings, are associated with the diagnosis of heart failure.2 Urgent clinical evaluation of patients presenting with dyspnea includes determining whether it is cardiac or respiratory in origin. Because the diagnosis is potentially difficult, a biochemical marker is useful.7 Since 2000, 2 assays have been available for the B-NPs, 1 for the active component, BNP, and 1 for the inactive fragment of the BNP precursor, NT-ProBNP.

B-NP testing initially was introduced as a probabilistic marker to determine whether dyspnea was of cardiac or respiratory origin.8,9 Currently, there is enough clinical evidence to unequivocally state that elevation in either BNP or NT-ProBNP indicates that respiratory distress/acute dyspnea is due to heart failure. This hypothesis has survived the test of numerous clinical studies, peer-reviewed literature, and evidence-based medicine reviews. The B-NPs have evolved in use from a probabilistic marker that discriminates between respiratory and cardiac causes of heart failure to a diagnostic marker of heart failure at the appropriate cutoffs for the particular assay.10,11

The B-NPs have also been studied for their use in diagnosing multiple other clinical conditions, as listed in **Box I**. Most studies used BNP instead of NT-ProBNP; however, relatively recent studies have demonstrated similar uses for NT-ProBNP.6,10 In addition, there are a number of clinical conditions not associated with cardiac disorders that have been reported to affect levels of BNP and NT-ProBNP **(Box II)**.

The current instrumentation manufacturer and the assay or assays the vendor dsupports are significant factors in an institution's choice of whether to assay BNP or NT-ProBNP. The major preanalytic, analytic, and physiologic differences between BNP and NT ProBNP are summarized in the Table. Omland and Hagve,1 Mair,4 Wu,12 and Yeo et al13 have recently published excellent biochemical and analytic reviews of issues associated with the NPs and the B-NPs. A significant supposed difference between BNP and NT-ProBNP is their respective relationship to renal function.

The complicated relationship between B-NP and renal failure has begun to be understood only in the past 2 years. Based on the initial understanding that B-NP was cleared extrarenally and NT-ProBNP was cleared renally, it had been presumed that BNP elimination was independent of GFR and that elimination of NT-ProBNP was entirely dependent on GFR. Studies published in the last 4 years have disproved those presumptions.[4,14,15] Few studies support the hypothesis of declining estimated GFR as the sole culprit for elevated NT-ProBNP.[16,17] In addition, 1 recent study has demonstrated that NT-ProBNP has a definitive extrarenal path of elimination.[14]

In a 2009 prospective study that compared BNP with NT-ProBNP levels in 165 hypertensive patients with a range of renal clearance rates, Van Kimmenade et al18 demonstrated the effect of renal function on B-NP clearance. Renal artery concentrations of NPs were compared with renal vein concentrations to determine the respective degrees of renal elimination as estimated GFR declined. Analysis of the results showed that BNP and NT-ProBNP were equally cleared by the kidneys, and that serum levels depend on renal function for both. Further analysis concluded that since both B-NPs depend on renal function, elevation of either in patients with heart failure was due both to cardiac disease and impaired renal clearance.

Both B-NPs are elevated in renal failure, but NT-ProBNP is elevated to a greater degree.[15] Initially, it was not known whether the NT-ProBNP elevation was due to a greater sensitivity to the renal component or if it was a more sensitive marker of early cardiac failure. Recent studies have shown that the elevation in NT-ProBNP, even in the face of renal failure, is due to congestive heart failure.[19] The effectiveness of NT-ProBNP testing, by itself and compared with BNP testing, in diagnosing heart failure is now being studied.

The ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study published its results in 2005 and 2006.[19,20] PRIDE was a prospective study of 600 patients who presented to the emergency department with dyspnea and were clinically diagnosed there as having heart failure independent of, and blinded from, NT-ProBNP results. NT-ProBNP levels less than 300 pg/mL ruled out heart failure, with a negative predictive value of 99%. Age-specific cutoffs for the diagnosis of heart failure (>450 pg/mL for patients younger than 50 years, and >900 pg/mL for patients 50 years or older) had sensitivities and specificities that were statistically significant. The study results indicated that NT-ProBNP level was superior to clinical judgment for the diagnosis of heart failure.

TABLE. *BNP vs NT-ProBNP*

	BNP	*NT-ProBNP*
Amino acids, No.	32	76
MW, kd	3.5	8.5
Hormonally active	Yes	No
Plasma half-life	20 min	60-120 min
Clearance	Clearance receptors Neutral endopeptidases Renal	Renal Systemic clearance, 14
In vitro stability	24 h at room temperature	>3 d at room temperature
Cross-reactivity	Nesiritide	Not reported
Specimen additive/tube	EDTA and heparin	Serum, EDTA and heparin, glass, or plastic
Units	pg/mL	pg/mL
Reference range, pg/mL†‡	Normal <100 Heart failure >500 recommended range	Normal<125 age <75 y Normal<450 age >75 y
BMI	Decreases	Decreases
Age	Mild increase	Significant increase
Antibody	Vendor dependent; C-terminal N-terminal or sequences	N-terminal and mid molecule sequences
Calibrator	Vendor specific but all calibrated to the same cutoff for BNP	Roche only
2006 CAP PT total enrollment	2932	476
2010 CAP PT total enrollment	3023	1169
2010 CAP PT enrollment, vendors	Abbott (all), 307 Beckman Coulter, 940 Biosite Triage, 1089 Siemens Advia (all), 687	Roche (all), 490 Siemens (Dimension, Vista and Stratus CS), 518 Vitros EIC,161

* Adapted from Wu.[12]
† BNP Reference ranges are standardized to each other.
‡ NT-ProBNP reference range is not interchangeable with that of BNP.
BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); BNP, brain natriuretic peptide; CAP PT, College of American Pathologists proficiency testing; and MW, molecular weight.

PRIDE data were further evaluated to see how NT-ProBNP vs BNP test results correlated with left ventricular function.21 Of the 209 patients with heart failure, left ventricular ejection fraction data were available for 153. From those data, the authors concluded that NT-ProBNP testing appeared to be superior to BNP testing for detecting heart failure in patients with preserved left ventricular ejection fraction. Other studies have shown NT-ProBNP to be a more sensitive and earlier marker of heart failure than BNP. [22-24]

Conclusion

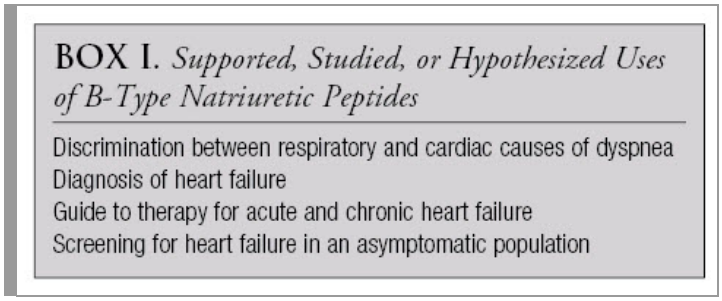
The usefulness of B-NP testing has rapidly evolved. Ten years ago, B-NP was a single marker used to probabilistically distinguish dyspnea due to cardiac causes from dyspnea due to respiratory causes. Today, BNP and NT-ProBNP are nearly definitive biochemical markers of heart failure. It has also been shown that both markers are eliminated by renal and extrarenal pathways. Furthermore, evidence is accumulating that the elevations in B-NPs seen in renal failure are not false-positives but subtle, early indications of heart failure.

Case Summary

The clinical pathologist's report is given below:

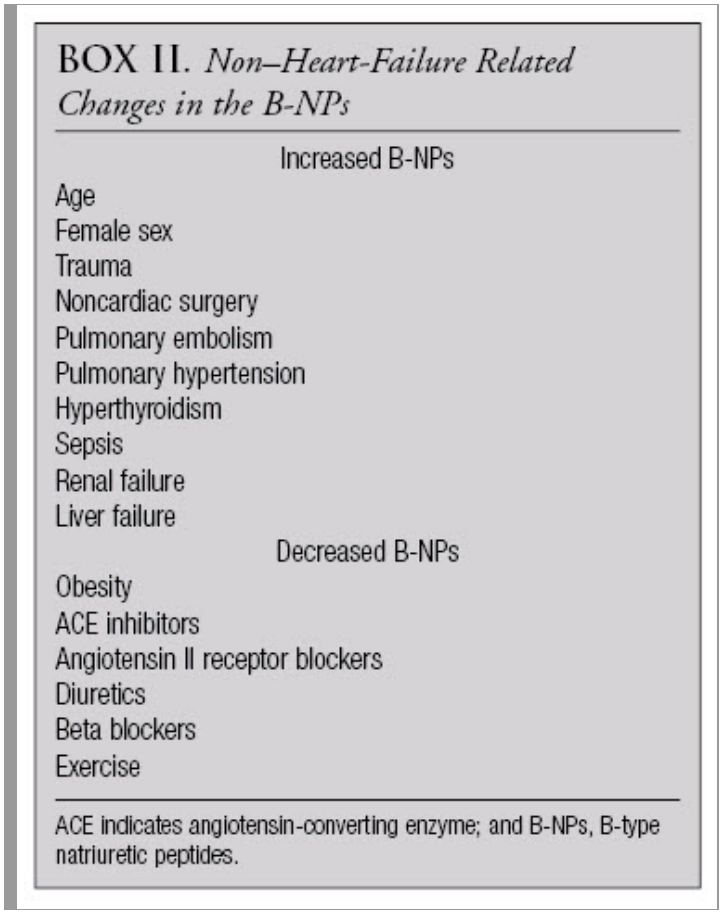
B-NP is a well-established, identified, and isolated counterregulatory cardiac hormone that is released in response to the body's compensatory mechanisms for heart failure. BNP and NT-ProBNP are 2 major fragments of B-NP; both are well-established commercial biomarkers of heart failure. BNP is active physiologically and NT-ProBNP is inactive, but they are very similar in their clinical utility. Any differences are related to preanalytic and analytic issues more than to interpretation. Clinically, they both are affected by declining renal function as well as worsening

heart failure; however, research is beginning to demonstrate that NT-ProBNP is superior to BNP in detecting subtle heart failure. Based on my review, there are still more similarities between the 2 markers than there are differences. Should the marker have to be changed to accommodate a change in instruments, I predict no adverse patient outcome, provided baselines are established for current patients, reference-range cutoffs modified, and a major communication process implemented to make all clinicians aware of the changes.



BOX I. *Supported, Studied, or Hypothesized Uses of B-Type Natriuretic Peptides*

Discrimination between respiratory and cardiac causes of dyspnea
Diagnosis of heart failure
Guide to therapy for acute and chronic heart failure
Screening for heart failure in an asymptomatic population



BOX II. *Non–Heart-Failure Related Changes in the B-NPs*

Increased B-NPs
Age
Female sex
Trauma
Noncardiac surgery
Pulmonary embolism
Pulmonary hypertension
Hyperthyroidism
Sepsis
Renal failure
Liver failure
Decreased B-NPs
Obesity
ACE inhibitors
Angiotensin II receptor blockers
Diuretics
Beta blockers
Exercise

ACE indicates angiotensin-converting enzyme; and B-NPs, B-type natriuretic peptides.

KEY TO IMAGES

Image 1 Box I
Image 2 Box II

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