

3/26/04 NACB Guide – Version 3

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Chapter 3, Part B: Analytical: Biomarkers in Heart Failure

Overview of Topic

As BNP and NT-proBNP become more heavily integrated into clinical practice as diagnostic and prognostic biomarkers, understanding differences between individual assay characteristics become extremely important. Further, the influence of both clinical, analytical and preanalytical factors on the growing number of BNP and NT-proBNP assays commercially available, begs for a better understanding by clinicians on how to interpret findings of different studies predicated on BNP or NT-proBNP concentrations monitored by different assays. The Laboratory Medicine community must also work closely with the in-vitro diagnostics companies to assist in defining the numerous assay characteristics, a process that were poorly orchestrated during the developmental phase of cardiac troponin testing. When BNP or NT-proBNP assays are used as biomarkers for diagnosis and prognosis, or used in clinical trials or studies, they should be well characterized, as suggested in the list of recommendations that follow. We recommend that when designing studies that will use BNP or NT-proBNP, investigators should review the STARD (Standards for Reporting Diagnostic Accuracy) (1) initiative for both assay characterization issues as well as for clinical and patient enrollment issues when monitoring BNP or NT-proBNP.

A growing diversity of BNP and NT-proBNP assays are used worldwide, emphasizing the need for both analytical and clinical validation of all commercial assays to support

definite clinical acceptance of these new biomarkers. At present, three FDA cleared BNP assays (Biosite, Bayer, Abbott) and one FDA cleared NT-proBNP assay (Roche) are commercially available for general clinical laboratory use. In addition, Beckman-Coulter has received FDA clearance to perform the Biosite BNP assay on their platform (Access). Research and development is also in progress towards release of multiple NT-proBNP assays using Roche reagents on multiple platforms (Dade-Behring, DPC). The number of assays will only continue to grow, making it even more essential that appropriate clinical and analytical assay criteria are uniformly adapted. The accurate clinical performance of each BNP or NT-proBNP assay, which may serve as the basis for life and death medical decisions, sets the stage to establish assay criteria as indispensable.

BNP and NT-proBNP are determined by a number of different immunoassays using antibodies directed to different epitopes located on the antigen molecules. For BNP one antibody binds to the ring structure and the other antibody to either the carboxy- or amino-terminal end. Degradation of BNP (a.a. 77 to 108) is known to occur by proteolytic cleavage of serine and proline residues in-vivo and in-vitro (2,3). This degradation may effect antibody affinities and thus be responsible for differences in stabilities of BNP monitored by different commercial BNP assays. For NT-proBNP (a.a.1-76) monitoring, an improved understanding of potential crossreactivity with split products of the N-terminal portion of NT-proBNP and proBNP itself are needed. For both assays, BNP and NT-proBNP, minimizing interferents from heterophilic antibodies and rheumatoid factor, for example, need to be established. The influence, stabilizing or destabilizing, of anticoagulant additives as well as the type of collection tube, have been well described (2,4). For BNP, EDTA anticoagulated whole blood or plasma appears to

be the only acceptable specimen choice. Presently, only one system, the Biosite Triage meter, allows for the direct measurement of whole blood (EDTA) BNP. For NT-proBNP, serum, heparin plasma, and EDTA plasma (reads 10% lower) appears acceptable. Presently, no whole blood assay is commercially available.

In the clinical setting, BNP and NT-proBNP assay characteristics need to be better understood or better established for optimal consideration as diagnostic and prognostic biomarkers. The influence of age, gender, ethnicity, and non-HF pathologies have been shown to substantially influence what may otherwise be considered a normal reference concentration (5). As an example, renal impairment has been shown to substantially increase NT-proBNP concentrations and BNP to a lesser extent (6,7). Obesity has also been shown to have an impact on BNP measurements; there is an inverse relationship between BMI increase and BNP decreases (8). As an example of confounding by treatment received, HF patients may receive the drug Nesiritide (human recombinant BNP) for therapy and management. Nesiritide is molecularly no different than endogenously released BNP. Thus, if BNP were to be monitored for regulation of Nesiritide infusion within a time window before an appropriate decrease of BNP could occur (half-life~22 minutes), the potential for false increased concentrations could arise. Nesiritide does not confound NT-proBNP measurements, however. Finally, a lack of understanding of the physiological and biological variability of BNP and NT-proBNP in HF patients may cause clinicians to misinterpret changing (increasing or decreasing) BNP and NT-proBNP concentrations in the context of establishing the success or failure of therapy. It has been shown that both BNP and NT-proBNP exhibit a intraindividual

biological variability of 35 to 45%. This translates to 100% when considering what is significantly different between serial BNP or NT-proBNP monitoring (9). A small study has also demonstrated that in HF patients monitored for BNP over at least 2 time periods during a 2 week period for BNP, that < 50% of concentrations were found to be outside the biological variability (10). This implies that BNP or NT-proBNP monitoring may be over used, and reemphasizes its role as a confirmation biomarker and not a stand alone test that clinicians should solely rely upon to manage HF patients.

Presently, there is no data available that have established a correlation between BNP and NT-proBNP concentrations using commercially available technology. In addition, the literature is scattered with home-brewed BNP and NT-proBNP assays, that may add to the confusion of clinicians when interpreting and comparing data from different studies; whether in diagnosing or ruling out HF, managing HF, screening for asymptomatic left ventricular dysfunction, or for risk stratification and prognostication for patients with HF, ACS or other pathologies. One must consider the assay used, the clinical evidence available based on the individual assay, and the aim of the biomarker based studies. No two assays are analytically equivalent at present. Thus until large studies are available, caution is suggested, before the conclusions based on one BNP or NT-proBNP assay study are translated to another.

RECOMMENDATIONS:

1. Assay dependent decision limits (median plus 95% CIs) correlated to NYHA Stages I through IV are recommended for optimal use of the natriuretic peptides BNP and NT-proBNP in confirming the diagnosis and severity of heart failure.

Strength/Consensus of recommendation: Class II b

2. Normal reference limits (95th or 97.5th percentile) should be independently established for both BNP and NT-proBNP based on age (by decade) and by gender. Each commercial assay should be validated separately.

Strength/Consensus of recommendation: Class IIa

The effect of ethnicity needs to be evaluated as a possible independent variable.

Strength/Consensus of recommendation: Class I c

3. Both BNP and NT-proBNP assay are acceptable for monitoring patients with the suspected diagnosis of heart failure. Simultaneous monitoring of both biomarkers is not warranted.

Strength/Consensus of recommendation: Class I c

4. ROC curves should be established to evaluate the clinical effectiveness and to establish optimal decision cutoffs for both BNP and NT-proBNP assays for diagnostic usefulness.

Strength/Consensus of recommendation: Class II b

5. Early in the research and development process, manufacturers should seek guidance and provide support to professional organizations within Laboratory Medicine, Cardiology, and Emergency Medicine to establish committees to initiate standardization of BNP and NT-proBNP between different assays of the same analyte, and to assist in establishing acceptance of preanalytical characteristics.

Strength/Consensus of recommendation: Class I c

6. The laboratory should perform, ASAP, BNP or NT-proBNP testing on a continuous 24 hour basis, with a TAT of <60 minutes. The TAT is defined as the time from blood collection to notification of result to physician or caregiver. Either central laboratory instrumentation or POC testing systems are acceptable.

Strength/Consensus of recommendation: Class II c

7. Assays for BNP and NT-proBNP should have a total imprecision (%CV) of $\leq 10\%$ at both their age and gender defined upper reference limits as well as at the NYSHA defined medical decision concentrations.

Strength/Consensus of recommendation: Class II c

8. Before introduction into clinical practice, BNP and NT-proBNP assays must be characterized with respect to the following preanalytical and analytical issues.

Preanalytical:

- a) effect of storage time and temperature
- b) influence of different anticoagulants

c) influence of gel separator tubes

Analytical

a) identification of both antibody recognition epitopes

b) crossreactivity characteristics with related natriuretic peptides, including NT-proANP, ANP, CNP, BNP, NT-proBNP, proBNP, etc.

c) identification of interferences from heterophile antibodies, rheumatoid factors, human anti-mouse antibodies

d) description of calibration material used and how the material was defined

e) clarification of dilution responds

Strength/Consensus of recommendation: Class I c

9. For both BNP and NT-proBNP, until a primary reference material is defined for either assay for appropriate calibration of assays, measurements should be reported in pg/mL units, not pmol/mL.

Strength/Consensus of recommendation: Class II c

10. For both BNP and NT-proBNP biological variability has been defined as being approximately 100%. Therefore, caution should be exercised in interpreting <100% concentration changes as being related to medical therapy regarding decreasing or increasing blood concentrations.

Strength/Consensus of recommendation: Class I b

11. As numerous clinical factors, such as renal function, obesity, and thyroid function are known to affect both BNP and NT-proBNP concentrations, appropriate reference intervals in these populations, without known HF, should be established.

Strength/Consensus of recommendation: Class I c

12. No primary reference materials are validated for calibration of BNP or NT-proBNP assays. As more commercial systems are introduced into the marketplace, patients specimen comparisons and regression analysis should be performed, along NCCLS guidelines, to establish the degree of or lack of harmonization across the dynamic range of each assay. Specifically, harmonization around the current presumed optimal diagnostic medical decision cutoff of 100 pg/mL for BNP should be validated. Since there is only one source of antibodies and calibrators for NT-proBNP (Roche), harmonization of NT-proBNP assays should not be a problem. This information is essential for clinical use when multiple assays are utilized in clinical studies/trials for diagnostic or risk stratification in HF or other pathologies relying upon BNP or NT-proBNP for independent clinical interpretation. Understanding that different BNP and NT-proBNP assay concentrations may vary several fold will aid in the critical review and understanding of each assay's clinical performance.

Strength/Consensus of recommendation: Class I c

References:

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