National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical Issues for Biomarkers of Heart Failure

WRITING GROUP MEMBERS
Fred S. Apple, PhD; Alan H.B. Wu, PhD; Allan S. Jaffe, MD; Mauro Panteghini, MD, PhD; Robert H. Christenson, PhD

NACB COMMITTEE MEMBERS
Robert H. Christenson, PhD, Chair; Fred S. Apple, PhD; Christopher P. Cannon, MD, Boston, Mass; Gary Francis, MD, Cleveland, Ohio; Robert L. Jesse, MD, PhD; David A. Morrow, MD, MPH, Boston, Mass; L. Kristen Newby, MD, MHS, Durham, NC; Alan B. Sorrow, MD, Nashville, Tenn; W.H. Wilson Tang, MD, Cleveland, Ohio; Alan H.B. Wu, PhD

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Fred S. Apple, PhD, Chair; Robert H. Christenson, PhD; Allan S. Jaffe, MD; Franca Pagani, MD, Brescia, Italy; Jillian Tate, MS, Brisbane, Australia; Jordi Ordonez-Llanos, MD, PhD, Barcelona, Spain; Johannes Mair, MD, PhD, Innsbruck, Austria

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I. Overview of Analytical Issues for Heart Failure Biomarkers

A. Background
In 2005, the IFCC C-SMCD recommended analytical and pre-analytical quality specifications for natriuretic peptide and their related co-metabolites assays. The objectives developed were intended to guide manufacturers of commercial assays and clinical laboratories that utilize these assays. The overall goal was to establish uniform criteria so that the analytical and clinical performance of assays natriuretic peptide and their related co-metabolites could be evaluated objectively. As B-type natriuretic peptide (BNP)
and N-terminal proBNP (NT-proBNP) become more heavily integrated into clinical practice as diagnostic and prognostic biomarkers, understanding the differences between individual assays becomes important. Further, the influence of clinical, analytical and preanalytical factors on the growing number of BNP and NT-proBNP assays commercially available begs for a better understanding of 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 all of the assay characteristics, a process that was poorly orchestrated during the developmental phase of cardiac troponin assays. When BNP or NT-proBNP assays are used as biomarkers for diagnosis, therapy decisions, and prognosis, or used in clinical trials or studies, they should be well characterized, as suggested by the list of recommendations that follow. We recommend that when designing studies that will use BNP or NT-proBNP assays, investigators should review the STARD (Standards for Reporting Diagnostic Accuracy) initiative for both assay characterization issues as well as for clinical study design and patient enrollment issues. We also advocate that both analytic and clinical assay validation studies, including reference (“normal”) interval studies, be published in detail in the peer reviewed literature. Assays that do not provide adequate information for evaluation should be used with caution. To our knowledge these recommendations are the first international recommendations addressing the analytical aspects of BNP and NT-proBNP for clinical use in heart failure.

II. Analytical Biomarker Issues

A. Issues Related to B-Type Natriuretic Peptide (BNP) and N-Terminal proB-Type Natriuretic Peptide (NT-proBNP) Measurement

Recommendations for Analysis of Biochemical Markers of Heart Failure

Class I

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

Preanalytical:
- a) Sample type; including type of biological sample: serum, plasma, whole blood; and type of specimen collection tubes
- b) Effect of storage time and temperature

Analytical:
- a) Identification of antibody recognition epitopes
- b) Description of calibration material used; with identification of source and the concentration value assignment. Until a clear determination of the clinically relevant molecules is established and a corresponding reference system is defined, results for both BNP and NT-proBNP should be reported in ng/L, rather than pmol/L
- c) determination of cross reactivity characteristics with related NPs, especially for BNP, NT-proBNP and proBNP, as well as for, atrial natriuretic peptide, NT-proANP, C-type natriuretic peptide
- d) evaluation of dilution response
- e) evaluation of interferences such as heterophile antibodies, rheumatoid factors, human anti-mouse antibodies (Level of Evidence: C).

2. Upper reference limits, at the 97.5th percentile of the reference value distribution, 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 (Level of Evidence: C).

3. Patients specimen comparisons and regression analysis should be performed, along CLSI (formerly NCCLS) guidelines, to establish the degree of or lack of harmonization across the dynamic range of each assay. Harmonization has been proposed around the current presumed optimal diagnostic medical decision cutoff for heart failure of 100 ng/L for BNP, as found in the Breathing Not Properly Trial using the Biosite assay. This may not be ideal for other non-heart failure clinical situations. More formal harmonization efforts might well be necessary along the lines done for other analytes, ie, cardiac troponin and creatine kinase MB. Since there is only one source of antibodies and calibrators for NT-proBNP (Roche), harmonization of NT-proBNP assays should not be a problem (Level of Evidence: C).

4. ROC curves should be established to evaluate the clinical effectiveness and to establish optimal medical decision cutoffs for both BNP and NT-proBNP assays for diagnostic usefulness. Data need to be reported in concentration numbers to allow for consensus between assays and not only in quartiles and tertiles (Level of Evidence: C).

Class IIa

1. Assays for BNP and NT-proBNP should have a total imprecision (%CV) of ≤15% at concentrations corresponding to their age and gender defined upper reference limits (Level of Evidence: C).

2. The effect of ethnicity needs to be evaluated as a possible independent variable (Level of Evidence: C).

3. Caution should be exercised in interpreting <50% concentration changes as being related to medical therapy because a consistently high biological variation for both BNP and NT-proBNP exists. However, consistent trends should be followed as clinically important (Level of Evidence: B).

1. Scope of BNP and NT-proBNP Assays

The growing diversity of BNP and NT-proBNP assays used worldwide emphasizes the need for both analytical and clinical validation of all commercial assays prior to the clinical acceptance of these new biomarkers. At present, four companies (Biosite, Bayer, Abbott, and Beckman Coulter using Biosite reagents) have BNP assays cleared by the Food and Drug Administration (FDA) and four companies have FDA cleared NT-proBNP assays (Roche, Dade Behring, Ortho-Clinical Diagnostics, and Nanogen; all using Roche antibodies and calibrator material); with Response Biomedical (a point of care assay) available in Japan. Research and development is also in progress toward release of additional
NT-proBNP assays using Roche antibodies and calibrator material on both central laboratory platforms (Siemens) as well as point of care (POC) platforms (bioMerieux, Mitsubishi Kagaku Iatron, Inverness Medical, Radiometer). 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 recommendations for assay criteria as indispensable.

2. Biological Implications for Assays of BNP and NT-proBNP

BNP and NT-proBNP concentrations are determined by various 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. Both glycosylation and degradation of BNP (amino acid residues 77 to 108) is known to occur by proteolytic cleavage of serine and proline residues at the amino-terminal end in vivo and in vitro.1,4,5,8 Both processes may effect BNP recognition by antibodies and thus be responsible for differences in stabilities of BNP measured by different commercial BNP assays.6 Experimental observations have shown that proBNP, the precursor peptide that splits into BNP and NT-proBNP, cross reacts with commercial BNP assays.7,8,9 For NT-proBNP (amino acid residues 1-76) measurement, an improved understanding of potential crossreactivity with split products of NT-proBNP and proBNP (amino acid residues 1-108) itself are needed, as preliminary evidence demonstrates cross reactivity of proBNP in an NT-proBNP assay.8,10 For both BNP and NT-proBNP assays blocking antibody strategies minimizing interferences from heterophilic antibodies and rheumatoid factor, for example, need to be described.

3. Specimen Collection for BNP and NT-proBNP Measurement

The stabilizing or destabilizing influence of anticoagulant additives, as well as the type of collection tube, have also been addressed.11,12 For BNP, EDTA anticoagulated whole blood or plasma appears to be the only acceptable specimen choice. Presently, only the Biosite Triage and Abbott’s Point-of-Care i-STAT allow for the direct measurement of whole blood (EDTA) BNP. Samples should ideally be collected in iced tubes and processed rapidly to avoid in vitro degradation. For NT-proBNP, serum or heparin plasma is the specimen of choice on the larger instruments in clinical laboratories. EDTA plasma gives a consistent negative bias (8% to 10%) compared with matched serum samples for NT-proBNP. At least four whole blood assays (Roche Cardiac Reader, Dade Behring Stratus CS, Synx Pharma [Nanogen] StatusFirst, and Mitsubishi Pathfast) are commercially available for NT-proBNP determination. Blood collected in plastic tubes is necessary for BNP, while for NT-proBNP, either glass or plastic are acceptable. For proBNP a research assay has been developed.7

4. Clinical Impact of BNP and NT-proBNP Metabolism

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. Recent observations report that proBNP appears to show cross reactivity with at least the Biosite and Bayer BNP assays,7,8 conflicting with a report demonstrating that neither the Biosite or Shionogi BNP assays detect proBNP.13 This may explain why at least one study describes difficulty for detecting BNP (amino acid residues 77-108) in plasma of patients with severe heart failure and increased BNP concentrations by Biosite assay, when a non-immunologic measurement approach (ie, liquid chromatography (LC)-electrospray ionization Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry) was used.14 Release of intact proBNP in its glycosylated and deglycosylated forms in blood may, therefore, have substantial implications regarding clinical utilization of BNP and NT-proBNP assays.7,8,9,10,13

5. Other Effects and Considerations for BNP and NT-proBNP Values

The influence of age, gender, ethnicity, and non-HF pathologies have been shown to substantially influence what may otherwise be considered a physiological concentration.15,16 Renal impairment has been shown to increase NT-proBNP concentrations and increase BNP to a lesser extent.17–19 Obesity has also been shown to have an impact on BNP and NT-proBNP concentrations, with an inverse relationship between body mass index (BMI) and BNP and NT-proBNP concentrations in patients with and without CHF.20–22 It appears some of this variability is related to lean body mass, perhaps as a manifestation of testosterone metabolism. It appears that androgens reduce BNP and NT-proBNP levels.23 HF patients who receive the drug nesiritide (Natracor, human recombinant BNP) for therapy and management may have confounding BNP results, since nesiritide is molecularly identical to endogenously released BNP. Thus, if BNP concentrations were to be monitored for regulation of nesiritide infusion within a time window before an appropriate decrease of BNP could occur (theoretical half-life ∼22 minutes), the potential for false increased concentrations could arise. Conversely, Nesiritide does not directly confound NT-proBNP measurements. Changes in NT-proBNP in response to nesiritide have not been marked in most studies.24,25 Finally, a lack of definitive understanding of the biological variability of BNP and NT-proBNP 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. Both BNP and NT-proBNP have been shown to exhibit a high intra-individual biological variability.26–29 Thus when considering what is significantly different between serial BNP or NT-proBNP concentrations for clinical use, a change of approximately 85% for increases and 46% for decreases could at minimum be necessary. This implies that changes in BNP or NT-proBNP concentrations must be used cautiously and reemphasizes their role as confirmation biomarkers and not as stand alone tests that clinicians should solely rely on to manage HF patients.
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 clinical studies. To avoid misinterpretation of results, one must consider the assay used, the available clinical evidence based on that individual assay, together with the clinical aim of an individual biomarker based study. Due to the lack of a single molecular natriuretic peptide or metabolic entity in the serum, plasma or whole blood matrix tested and the cross-reactivity of the antibodies used toward these various NP forms, results for both BNP and NT-proBNP should be reported in ng/L, rather than pmol/L. No peer-reviewed literature has demonstrated that two NP assays are analytically equivalent. Until large studies are available, caution is suggested before the conclusions based on one BNP or one NT-proBNP assay-based study are translated to another assay-based context. Indeed, studies directed toward different clinical populations will often have very different cutoff concentrations. A synthesis of the rule in and rule out cutoffs for each clinical scenario is needed for the heart failure field to advance.30

References
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