Quality Specifications for B-Type Natriuretic Peptide Assays

Fred S. Apple,1* Mauro Panteghini,2 Jan Ravkilde,3 Johannes Mair,4 Alan H.B. Wu,5 Jillian Tate,6 Franca Pagani,2 Robert H. Christenson,7 and Allan S. Jaffe,8 on Behalf of the Committee on Standardization of Markers of Cardiac Damage of the IFCC

Background: The objective of this report is to improve the quality of immunochemical measurements of B-type natriuretic peptide (BNP) and its N-terminal propeptide (NT-proBNP). The recommendations proposed are intended for use by manufacturers of commercial assays, by clinical laboratories using those assays, by clinical trial groups and research investigators, and by regulatory agencies, such as the United States Food and Drug Administration.

Methods: A group of cardiac biomarker experts reviewed and abstracted the scientific literature to provide recommendations pertaining to the quality specifications for BNP/NT-proBNP assays.

Results: The evidence-based recommendations encourage manufacturers to endorse and consistently follow the proposed recommendations; encourage that all package inserts for BNP/NT-proBNP immunoassays include uniform information on assay design, preanalytical performance characteristics, analytical performance characteristics, and clinical performance; and encourage regulatory agencies to adopt a minimal and uniform set of criteria for manufacturers to provide when seeking clearance for new and/or improved assays.

Conclusions: These recommendations address the use of BNP and NT-proBNP as cardiac biomarkers and not their physiologic and/or pathophysiologic relevance.

*Address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories P4, 701 Park Ave., Minneapolis, MN 55415. Fax 612-904-4229; e-mail fred.apple@co.hennepin.mn.us. Received October 27, 2004; accepted December 14, 2004. Previously published online at DOI: 10.1373/clinchem.2004.044594

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The objective of this report is to improve the quality of immunochemical measurements of B-type natriuretic peptide (BNP) and its N-terminal propeptide (NT-proBNP; Table 1). The recommendations proposed are intended for use by manufacturers of commercial assays, by clinical laboratories using those assays, by clinical trial groups and research investigators, and by regulatory agencies, such as the United States Food and Drug Administration (FDA). The main objectives are as follows:

- For manufacturers to endorse and then consistently follow the proposed recommendations;
- To encourage that all package inserts for B-type natriuretic peptide immunoassays include this uniform information on assay design, preanalytical performance characteristics, analytical performance characteristics, and clinical performance characteristics as outlined below;
- To encourage publication of these quality specifications for each assay in peer-reviewed journals; and
- To encourage regulatory agencies to adopt a minimal and uniform set of criteria (specifically those suggested herein) for manufacturers to provide when seeking clearance for new and/or improved assays. These rec-

Nonstandard abbreviations: BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; FDA, United States Food and Drug Administration; NP, natriuretic peptide; HAAA, human anti-animal antibody; ANP, atrial natriuretic peptide; NPR, natriuretic peptide receptor; and NEP, neutral endopeptidase.
Table 1. Nomenclature for the NPs discussed in this report.

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<tr>
<td>pre-proBNP</td>
<td>Cellular precursor synthesized in the myocardial cell, containing 134 amino acids, including a signal peptide of 26 amino acids; present only in myocardial tissue</td>
</tr>
<tr>
<td>proBNP</td>
<td>Contains 108 amino acids (1–108); produced from pre-proBNP by cleavage of the signal peptide, when appropriate signals for hormone release are given; present in both myocardium and plasma</td>
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<tr>
<td>BNP</td>
<td>Biologically active hormone; corresponds to the C-terminal fragment of proBNP (amino acids 77–108); present in both myocardium and plasma</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>Entire N-terminal fragment of proBNP (amino acids 1–76), lacking hormonal activity; present in both myocardium and plasma; further degradation products of this molecule are sometimes identified with the same abbreviation [e.g., NT-proBNP (amino acids 1–21)], but little metabolic and pathophysiologic information is available for these molecules</td>
</tr>
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ommendations address the use of natriuretic peptides (NPs) as markers and not their physiologic and/or pathophysiologic relevance.

Background

Analytical validation studies of B-type natriuretic peptide assays tend to be designed to allow an assay to pass minimum regulatory criteria. Often this means simply showing equivalent analytical (as well as clinical) performance to an assay that already has FDA clearance. However, the studies used to establish reference limits, medical decision cutoffs, and analytical characteristics (e.g., total assay imprecision) often fail to adequately address the analytic and clinical needs of an assay to allow for consistent clinical interpretation across all patient subsets. The problem is attributable in part to the lack of accepted minimum quality specifications for new assays that are uniformly followed. Thus, new or revised cardiac biomarker assay development often leads to commercially available assays that lack method harmonization and hence create confusion for clinicians, laboratorians, and patients because of differences in assay characteristics. These issues are highlighted when attempting to compare results of multiple assays that measure the same analyte. Two laboratory medicine working groups, the IFCC Medicine Committee on Standardization of Markers of Cardiac Damage and the National Academy of Clinical Biochemistry committee on Cardiac Biomarkers, are promoting evidence-based recommendations. The purpose of these recommendations is to guide publications in peer-reviewed journals that will provide a consistent template within which companies can clarify analytic and practice issues. It would be ideal if regulatory agencies, such as the US FDA, also accept these criteria for 510(K) and premarket approval clearance applications. We suggest that only after appropriate analytical quality specifications are addressed that the many issues pertaining to (a) clinical interpretation of NP concentrations and (b) methodologic differences that lead to nonharmonized concentration values will be reconciled. Overall, our goal is to facilitate better patient care through accurate testing and believable and understandable results. The responsibility of defining and implementing these issues, as outlined here, must be a shared responsibility among laboratorians, industry, clinicians, and regulatory agencies on an international front.

Analytical Issues

CALIBRATOR CHARACTERIZATION

Definitive information about the synthesis and catabolism of proBNP, NT-proBNP, and BNP is needed to determine the characteristics of the calibrator(s) to be used in commercial assays. It is known that BNP derives from the pre-proBNP, which contains a signal peptide sequence at the N-terminal end. After the signal peptide is cleaved, proBNP is further split proteolytically into an inactive N-terminal fragment and the biologically active peptide hormone, BNP. The site at which proteolytic cleavage of proBNP takes place is still being debated. Most of it occurs within or on the surface of cardiomyocytes before the secretion in blood, but small amounts of intact proBNP are also found in the circulation, indicating that some proteolysis may occur in the circulation. These facts have been shown most clearly in the study of Hunt et al. (1), which compared the amounts proBNP-derived molecular forms found in cardiac tissue and in plasma. Qualitatively, the same peaks were seen after HPLC fractionation of an atrial extract as were seen in plasma from a patient with heart failure. BNP, proBNP, and NT-proBNP were found in both samples. The presence of intact proBNP in human plasma, in addition to BNP and NT-proBNP fragments, is important because of the potential that it may create problems regarding analytical specificity in the measurement of these peptides in plasma.

Another study demonstrated that the N-terminal region of proBNP contains a leucine zipper-like sequence motif that may induce peptide oligomerization in plasma under physiologic conditions, producing either a trimer or tetramer of proBNP and a trimer of NT-proBNP (2). These oligomerized molecules may expose or obscure epitopes recognized by the antibodies used in commercial assays.

Experimental data also support the cleavage of BNP by plasma proteases. Proteolysis of the C-terminal structure by kallikrein occurs after activation of the coagulation contact activation system by a negatively charged surface. This can occur in vivo on the intraluminal surface of a damaged vessel and/or in vitro on the glass wall of blood collection tubes (3). Proteolytic cleavage of the two N-terminal amino acid residues, serine and proline, may occur immediately after blood collection or within the circulation, making the N-terminal residue of BNP more sensitive to degradation (4). It may be critical to take these enzymatic cleavages, particularly the one at the NH₂
terminus, into account when choosing epitopes for antibody production and immunoassay design. Finally, a recent study has shown that circulating NT-proBNP is heterogeneous and that most immunoreactive NT-proBNP is significantly smaller in size than NT-proBNP 1–76 because of truncation at both termini (5). This fragmentation was more pronounced in serum than in plasma.

To obviate the NT-proBNP heterogeneity in plasma, Goetze et al. (6) developed a processing-independent analysis for quantification of proBNP and its fragments in plasma. Calibrators were prepared from synthetic tyrosine-extended proBNP amino acids 1–10. An antibody directed against amino acid sequence 1–10 of human proBNP was used, and before measurement, plasma was treated with a proteinase (trypsin) that cleaved all proBNP peptides to the amino acid 1–21 fragment. The clinical utility of this approach remains to be determined, but it would overcome the issue of differential detection of circulating proBNP-derived fragments by different assays (7). NT-proBNP (amino acids 1–76) also is difficult to prepare for use as a calibrator material; thus, shorter synthetic peptides with sequences encompassing the epitope region recognized by assay antibodies have frequently been preferred. Theoretically, for both BNP and NT-proBNP assays, there is the requirement for the composition of the calibrator to resemble that of the analyte present in the patient sample. However, because these peptides are heterogeneous and their composition in human body fluids may vary significantly, calibrator materials can be surrogates only for the analytes to be measured in patient samples. Although such materials may resemble to some extent the typical heterogeneous mixture of the analytes present in human fluids, in practice they may represent only an “average” condition. Definitive evidence, obtained by studying the differential release characteristics of peptides in response to diverse physiologic and pathologic stimuli and their clearance and degradation mechanisms, is needed to determine which peptide fragments are present in the circulation. In addition, these peptides/fragments need to be measured to obtain the greatest clinical utility, which may vary depending on the clinical situation.

Recommendations:

- Assays should be calibrated against the material representing the molecule showing the greatest clinical utility in a given clinical situation. For this type of definition, an evidence-based international agreement is required to:
  - Identify which NP fragments are present in the circulation by studying the differential release characteristics of NPs in response to diverse physiologic and pathologic stimuli; and
  - Identify which fragments should be measured to obtain the greatest clinical efficiency. This may vary depending on the clinical situation.

- Identify the clearance and degradation mechanism of all NP fragments present in the circulation.
- Determine whether detection by commercial assays is equimolar or nonequimolar for the fragments that exist in blood. Such an effort will require understanding the affinities of the antibodies used in commercial assays for the circulating NP fragments.
- Lacking traceability to SI units, the use of ng/L instead of pmol/L is recommended.

Information required:

- Characterization and source of calibrator material; i.e., animal or human, native or recombinant.
- Documentation of analyses performed to demonstrate the purity of and concentration assigned to the material.
- Description of calibration curve (e.g., curvi- or rectilinear response) and number of calibration points.

ASSAY SPECIFICITY

**BNP.** Commercially available assays cleared for measurement of BNP are sandwich-type immunoassay methods based on two monoclonal antibodies or a combination of monoclonal and polyclonal antibodies (Table 2). Usually one antibody binds to the ring structure, which is formed by a disulfide bond, and the other antibody to either the C- or N-terminal end of BNP, respectively. After its release into the circulation, BNP (amino acids 1–32) is degraded at the NH2 terminus to BNP 3–32 by proteolytic cleavage of serine and proline residues (8). This degradation may affect the affinities of antibodies that bind to epitopes at the N-terminal end of the peptide. No additional degradation products of BNP 1–32 or 3–32 have been found to date by HPLC analysis in plasma from heart failure patients (4). We cannot exclude the possibility that some conversion occurs in the sample per se. The disulfide-bond-mediated ring structure and the C-terminal structure appear to be stable in blood samples (4, 8). This does not mean that degradation can never occur, as reported by Belenky et al. (9). However, at present, there are no data to indicate that the antibodies to BNP do not capture and/or tag proBNP as well. If that does occur, it is also possible that such antibodies also could detect the multimers described by Shimizu et al. (4).

**NT-proBNP.** Two sandwich-type NT-proBNP immunoassays have been cleared by the FDA and worldwide for routine application at present (Table 2); several others are under development. Polyclonal antibodies detect the epitopes containing amino acids 1–21 and 39–50 on proBNP. In Europe, one additional competitive enzyme immunoassay is available. Its polyclonal antibodies are directed against epitope 8–29 on proBNP. To date, intact NT-proBNP (amino acids 1–76) has not been detected by HPLC analysis in plasma from heart failure patients.
Rather, HPLC analyses have revealed that assay immunoactivity is attributable to material that is smaller in size and that circulating immunoactive NT-proBNP is strongly heterogeneous (10). The immunoactivity probably represents NT-proBNP 1–76-derived peptides truncated at both termini (5). NT-proBNP assays theoretically should show 100% cross-reactivity with proBNP. Furthermore, intact proBNP has been described in human plasma, and oligomerization probably occurs (11).

Assays for BNP and NT-proBNP may differ in their susceptibility to analytical interferences. Interferences from heterophilic antibodies, such as rheumatoid factors, or from human anti-animal antibodies (HAAAs) may lead to false test results (12). Formulation of immunoaassays requires minimization of interference from heterophilic antibodies and HAAAs; addition of nonimmune serum from the animal species that was used to raise the antibodies is effective (12, 13). Icteric and hemolyzed samples might also be a problem in certain immunoassays with fluorometric detection of the signal. Some analyzers and other peptide hormones, including proBNP and its multimers.

Specifications of the type of nonimmune serum as a blocking agent to be added to the reagent formulation or alternative blocking agent used to neutralize the effect of heterophilic antibodies and HAAAs.

Description of the relevant interferences by high concentrations of endogenous constituents, such as hemoglobin, triglycerides, bilirubin, and paraproteins. A full description of the experimental and statistical design that was used to assess interference effects is recommended (13).

Recommendations:
- Assays should not cross-react with structurally related NPs [atrial natriuretic peptide (ANP), C-type NP, urodilatin, proANP, NT-proANP, fragments of NT-proANP] or other peptide hormones.
- BNP assays should demonstrate 100% cross-reactivity with BNP 3–32.
- As long as the metabolism and degradation processes for proBNP/NT-proBNP are not fully understood, no recommendation about which specific epitopes or regions on proBNP should be detected by the NT-proBNP assay antibodies can be provided. According to recent results, the most robust assays use antibodies directed at the central portions of NT-proBNP (5).
- Documentation of the extent of cross-reactivity between antibodies for BNP and proBNP and fragments related to proBNP and proBNP multimers is required for each BNP assay.
- The lack of interference from heterophilic antibodies and HAAAs in an assay system should be carefully documented by testing of samples containing high concentrations of rheumatoid factor or human antihuman antibodies (or appropriate anti-species antibodies). Measurements should be made before and after the addition of a blocking reagent or in conjunction with pretreatment of the sample with heterophilic antibody-blocking tubes.

**Information required:**
- Exact epitope characterization on the BNP/NT-proBNP peptides to which the assay antibodies bind.
- Cross-reactivity with BNP 3–32 for both BNP and NT-proBNP assays as well as cross-reactivity for all NPs and other peptide hormones, including proBNP and its multimers.
- Specifications of the type of nonimmune serum as blocking agent to be added to the reagent formulation or alternative blocking agent used to neutralize the effect of heterophilic antibodies and HAAAs.

**ANALYTICAL IMPRECISION AND DETECTION LIMITS**

The within-run and total imprecision values obtained with different commercial assays for BNP and NT-proBNP are not uniform. In general, assays on automated platforms perform better than manual or point-of-care tests (14–17). Irrespective of where the testing is performed (i.e., laboratory-based or near bedside), a decision concerning what is acceptable precision is needed. We

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**Table 2. Commercial BNP/NT-proBNP assays and antibody reactivities.**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Antigen(s)</th>
<th>Capture antibody</th>
<th>Detection antibody</th>
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<tbody>
<tr>
<td><strong>Abbott AxSYM BNP</strong></td>
<td>BNP amino acids 1–32; BNP amino acids 3–32; proBNP amino acids 1–108&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Scios (ring structure and possibly part of arm extending to COOH terminus)</td>
<td>Shionogi (COOH terminus; BC203)</td>
</tr>
<tr>
<td><strong>Bayer Centaur BNP</strong></td>
<td>BNP amino acids 1–32; BNP amino acids 3–32; proBNP amino acids 1–108&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Shionogi (ring structure; KYhBNP-II)</td>
<td>Shionogi (COOH terminus)</td>
</tr>
<tr>
<td><strong>Biosite Triage BNP</strong></td>
<td>BNP amino acids 1–32; BNP amino acids 3–32; proBNP amino acids 1–108&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Scios (ring structure and possibly part of arm extending to COOH terminus)</td>
<td>Biosite (NH₂ terminus)</td>
</tr>
<tr>
<td><strong>Shionogi IRMA BNP</strong></td>
<td>BNP amino acids 1–32; BNP amino acids 3–32; proBNP amino acids 1–108&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Shionogi (ring structure; KYhBNP-II; amino acids 14–21)</td>
<td>Shionogi (COOH terminus; BC203; amino acids 27–32)</td>
</tr>
<tr>
<td><strong>Roche Elecsys NT-proBNP</strong></td>
<td>NT-proBNP amino acids 1–76; proBNP amino acids 1–108&lt;sup&gt;a&lt;/sup&gt;; truncated NT-proBNP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Roche (NH₂ terminus; amino acids 1–21)</td>
<td>Roche (central molecule; amino acids 39–50)</td>
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<sup>a</sup>Possible cross-reactivity.
concur with the goals of the model proposed by Cotlove et al. (18), based on the concept that the effect of analytical imprecision should not significantly affect the clinical use of the biomarker. This model suggests that the desired low impact of imprecision can be obtained when the analytical CV is lower than or equal to one half the intra-individual biological variation so that the combined (analytical plus biological) CV does not increase by more than 12% compared with the intra-individual biological CV. Because a consistently high biological variation for both BNP and NT-proBNP, no doubt attributable to poorly understood physiology, has been reported in the literature (within-subject CV, 30–50%), very low assay imprecision may be unnecessary (19–21). However, for monitoring of therapy with serial NP measurements in clinical cases, it may be desirable to minimize the analytical imprecision component of the NP variations.

A concept closely related to imprecision is the limit of detection, defined as the lowest concentration of BNP or NT-proBNP that can be detected with a reasonable certainty of measurement for a given assay; i.e., the lowest value that can be taken to be different from zero. In general, the detection limit required depends on the clinical use of the measured biomarker. In the case of cardiac NPs, it should be significantly lower (four- to fivefold) than the reference limit obtained from a reference population of apparently healthy individuals.

Recommendation:

- A desirable total imprecision (CV) of <15% at BNP/NT-proBNP concentrations within the reference interval is recommended. If an eventual goal is to rely on monitoring of marker trends over time, then an optimal total imprecision of <10% would be advocated.

Information required:

- Definition of the imprecision profile, i.e., scattergraph showing on the ordinate the CV in percentage vs increasing NP concentrations on the abscissa. This should be obtained using the NCCLS EP5-A protocol by assessing pools of human serum, plasma, or whole blood samples containing different biomarker concentrations. Samples that encompass BNP and NT-proBNP concentrations within the reference intervals from patients with congestive heart failure must be included.
- Definition of detection limit, i.e., the NP concentration corresponding to a signal 3 SD above the mean (n = 20 within-run measurements) for a sample (zero calibrator) in which BNP and NT-proBNP are absent.

INTERNATIONAL STANDARDIZATION OF BNP IMMUNOAASSAYS

There is a lack of standardization of currently available NT-proBNP and BNP assays, as shown by differences in values for method comparisons of patient samples. 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 (22–25). Antibodies that recognize similar molecular form(s) of NT-proBNP or BNP in blood, in an equimolar manner, are required for standardization of methods. Use of standardized assays should produce the same true values for different methods and enables a sharing of common reference intervals and/or decision cutoffs for diagnosis and treatment.

Recommendations:

- Suitable reference materials, i.e., a primary standard and secondary serum-matrix-based reference materials should be available.
- Reference materials should be defined by a set of specific characteristics including the general properties of origin, mode of production, physical matrix, additives for preservation and stability, and optimum storage requirements.
- When intended for direct value assignment to manufacturers’ calibrators, certified reference materials must have properly documented characteristics and be compatible to the analyte in blood; e.g., exhibiting no matrix effect and other properly documented characteristics.

Preanalytical Issues

Data regarding the in vitro stability of BNP and related peptides are sparse and conflicting. Proteolytic degradation of the BNP molecule appears to occur as soon as blood is collected. Sample stability appears to be method dependent, evidently because of the different stabilities of epitopes targeted by different assays. Furthermore, BNP is reportedly unstable when collected in glass tubes because of activation of kallikreins of the extrinsic clotting pathways, but this phenomenon may be dependent on the specificities of antibodies used in the measurement method (3). NT-proBNP appears to be relatively stable during sample storage. For BNP assays, EDTA plasma is the only suitable specimen. At present, it appears that for the Elecsys NT-proBNP assay, serum is the matrix of choice. Differences between serum and plasma NP concentrations measured by different analytical systems have been detected. Consequently, the type of anticoagulant used should be studied and validated thoroughly before it can be recommended for practical use.

The NPs (A-, B-, and C-types) exert their effects through interactions of natriuretic peptide receptors (NPRs) found on the surfaces of target cells (26). Three receptors (identified as A, B, and C) have been isolated in a variety of human tissues. Binding of the NPs to NPR-A and -B stimulates intrinsic guanyl cyclase activity and the production of the intracellular messenger, cGMP. NPR-A receptors are most abundant in large vessels, whereas NPR-B receptors are found in the brain. NPR-C receptor
binds all NPs and is partly responsible for the clearance of these peptides from the circulation. Binding to the receptor leads to enzymatic degradation in situ and is a major mechanism for the clearance of these peptides from the circulation by endocytosis (27). Neutral endopeptidases (NEPs) have also been implicated in the degradation of the NPs (28). NEPs are zinc-containing metalloendopeptidases that cleave substrates on the amino side of hydrophobic amino acids. In the case of the NPs, they cleave the linkage between amino acids cysteine and phenylalanine in positions 10 and 11. NEPs are abundant in kidney brush border membranes, cardiac myocytes, and endothelial cells and can degrade other circulating peptides, such as kinins, enkephalins, and neurotensins. Although ANP is cleared by NEPs, this pathway does not appear to be as important for BNP (30), leading some to suggest that another, as yet unidentified pathway is important (29). However, NEPs are up-regulated in patients with renal failure.

Another mechanism for BNP clearance may be through glomerular filtration. Increased concentrations of BNP are observed in patients with chronic renal failure (31). NT-proBNP is biologically inactive and does not bind to NPRs, and it does not appear to be degraded by NEPs (10). Clearance of NT-proBNP is hypothesized to be most likely through renal excretion. Observations of markedly increased NT-proBNP concentrations in patients with renal failure suggest that the kidneys may be important for the clearance of NT-proBNP. However, increased NT-proBNP concentrations could be just an effect of volume overload, which is an important stimulus for BNP secretion. The circulation half-life of NT-proBNP is thought to be longer than that of BNP. It may also explain why the relationship between NT-proBNP and the estimated glomerular filtration rate has a tighter correlation than for BNP and estimated glomerular filtration rate (32). NT-proBNP and/or proBNP are found in the urine. Of interest, the amount detected increases as glomerular filtration rate diminishes (33).

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 1 month or, with addition of the protease inhibitor aprotinin, longer (34). Neurondopeptidases are not likely involved with in vitro degradation because BNP degradation continues after deactivation of NEPs by EDTA chelation. Therefore, in vitro BNP instability is most likely attributable to proteases in serum and plasma, as suggested by studies of BNP stability in the presence of protease inhibitors (9).

The NT-proBNP assay is relatively resilient to sample storage, and 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 (35). Samples are also stable for at least 1 year when stored at −80 °C, and five freeze–thaw cycles had no effect on analyte concentration (36). For the Roche assay, a small but statistically significant difference in results between heparin plasma and serum was shown in one study (37), but was not confirmed in others (35). EDTA plasma gave a consistent negative bias (6–10% on average) compared with matched serum and heparin-plasma samples, although the studies did not indicate the variability among samples (38).

Recommendations:

- When measuring BNP, blood should be collected only in plastic tubes containing EDTA unless specific assays are appropriately validated with glass tubes. When measuring NT-proBNP, blood should be collected for serum monitoring. Use of other anticoagulants must be based on comparisons among different kinds of samples (e.g., EDTA or lithium-heparin plasma vs serum).
- Specific assays must be appropriately examined for sample stability because there appears to be assay dependence. In the absence of these studies, BNP should be measured within 4 h of collection if the sample is stored at room temperature. If the testing cannot be performed within 4 h, the sample should be centrifuged and separated, a kallikrein- or serine-specific protease inhibitor should be added, and the plasma should be stored refrigerated at 4 °C for up to 72 h or frozen (ideally at −70 °C) if stored for longer periods.
- NT-proBNP is stable for at least 72 h at room temperature or refrigerated at 4 °C (16, 37).
- Each BNP and NT-proBNP assay needs to be validated for the effect of freeze–thaw cycles on analyte stability (36).

Information required:

- Sampling tube compositions must be specified. Manufacturer and tube catalog number should also be given.
- For plasma samples, the manufacturer’s package insert should document the type and concentration of anticoagulant usable, with a full description of the experimental and statistical design that has been used to assess possible anticoagulant effects.
- Information on maximum in vitro stability at different storage temperatures should be given for the sample materials recommended for each assay.
- The effect of gel separation barriers on BNP an NT-proBNP concentrations should be documented.

Clinical Importance

Because BNP and NT-proBNP are released mainly from the cardiac ventricles in response to increased stretch and wall tension, it is not surprising that increased plasma concentrations of these NPs have been described in congestive heart failure, asymptomatic left ventricular dysfunction, arterial and pulmonary hypertension, cardiac hypertrophy, valvular heart disease, arrhythmia, and acute coronary syndrome. Because BNP and NT-proBNP are increased in a variety of cardiac and noncardiac diseases, clinicians must be extraordinarily conscious in
interpreting a result. Furthermore, BNP and NT-proBNP may become components of a panel of biomarkers, along with cardiac troponin, to be used for risk stratification in acute coronary syndrome. Basic clinical questions not addressed in this report, i.e., determination of age-, gender-, and ethnicity-dependent reference values and cutoff values for diverse disease entities, must be addressed to allow for the appropriate clinical usefulness of BNP and NT-proBNP. Until many of the analytical and clinical issues are elucidated, it will be a challenge to support a universal cutoff concentration for use of these analytes for diagnostics, therapy guidance, or risk stratification.

Conclusions

Laboratorians and clinicians must be cognizant of the numerous considerations inherent in the NPs as markers for management of cardiology patients, including

- The form of the biomarker itself (BNP or NT-proBNP);
- The lack of standardization of immunoassays;
- That reference and medical decision limits are dependent on age and gender;
- That biological variation of NPs in individuals is inherently high;
- The diagnostic time window (admission or monitoring trends over time);
- The clinical setting in which NPs are used (e.g., general practice, emergency room, and coronary care unit);
- The patient subset being tested (i.e., renal failure, sepsis); and
- Whether application is for diagnostic use, prognostic use, or for a future potential application of therapeutic guidance.

All of these aspects must be taken into consideration with the implementation of biomarkers such as NPs to avoid the possibility for misinterpretation of a result for patient care.

References

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