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Troponin and BNP: Diagnostic laboratory testing in Cardiovascular Disease Management

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Abstract

Heart failure (HF) is a prevalent and costly syndrome resulting from structural or functional cardiac disorders that impair the heart's ability to maintain physiological circulation. Diagnosis relies on clinical judgment combining history, physical examination, and appropriate investigations, with natriuretic peptides, particularly B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP), serving as first-line biomarkers. BNP and NT-proBNP levels indicate treatment effectiveness and are strongly recommended for prognostic purposes. Elevated cardiac troponin levels, measured using high-sensitivity methods, are associated with worse clinical outcomes in HF patients. Additional biomarkers, such as galectin-3 and soluble ST2, have been suggested for risk stratification. A multi-marker approach combining multiple biomarkers is considered the most effective model for individual cardiovascular risk prediction. However, developing reliable and robust

measurements for cardiovascular biomarkers poses significant challenges due to their low concentrations and heterogeneity in circulation. Standardization efforts aim to establish reference measurement procedures and materials to enhance comparability and reliability. Harmonization, defined as a reduction in heterogeneity among results from different methods, may be attainable through deeper insights into the biochemical properties and pathophysiological roles of candidate biomarkers. The B-type cardiac natriuretic peptide system exemplifies the complexity of biomarker measurement, with numerous proBNP-derived fragments identified in human plasma. Developing more specific assays for active BNP1–32 and concurrent measurement of proBNP and BNP could improve harmonization and understanding of cardiac endocrine dysfunction in HF. Rigorous evaluation of new methods in clinical trials, following evidence-based principles, is essential to determine their utility compared to existing assays.
Keywords: Troponin, BNP, Diagnostic laboratory testing, Cardiovascular Disease, CVD

Introduction

Estimates suggest that the prevalence of symptomatic heart failure (HF) in the general population of Europe and North America ranges from 0.4% to 2% (Heart Failure Society Of America, 2010). The incidence and prevalence of HF increase significantly with age, nearing 1 in 1000 individuals among those over 65 years of age. Economically, HF represents a major burden, being the leading expenditure for Medicare in the United States and a significant cost driver in healthcare systems across European nations. Despite significant advancements over the last five decades in the understanding and treatment of HF, the prognosis remains poor. Approximately 40% of patients with severe HF (classified as NYHA class III–IV or ACC/AHA stage D) in Europe and North America succumb within one year of diagnosis. The survival rates are comparable to those observed in colon cancer and worse than those for breast or prostate cancer (Nieminen et al., 2005).

Nearly two decades ago, Braunwald and Bristow proposed the hypothesis that HF, traditionally deemed irreversible and only manageable through palliative care, might, in fact, be reversible. They suggested that intrinsic myocardial contraction defects in some chronic HF patients could be partially reversed using ventricular assist devices over several months (Vittorini & Clerico, 2008) and/or with appropriate pharmacological treatment. It has since been well-documented that patients with chronic HF treated with β-adrenergic blockers, in addition to ACE inhibitors, show improved systolic function and potential reversal of cardiac remodeling. This treatment approach correlates with improved clinical outcomes, including reduced hospitalizations and extended survival. Consequently, the perception of chronic HF as an irreversible, end-stage disease is evolving into a concept where intrinsic functional and structural defects of the failing heart can be addressed with suitable therapy. Theoretically, intervening during the early stages of cardiac dysfunction is more likely to arrest or even reverse the progressive nature of HF.

To underscore the importance of both disease development and progression, the ACC/AHA guidelines categorize HF into four stages, from A to D. Stages A and B encompass asymptomatic patients, aiming to highlight the importance of early identification of individuals at risk for HF. Stage A patients exhibit only risk factors without structural or functional myocardial alterations, whereas stage B patients display cardiac structural changes (e.g., hypertrophy) or functional impairments (e.g., reduced left ventricular function). Stages C and D represent symptomatic patients. Advances in identifying risk markers and specific disease indicators have led to the proposal of new cardiovascular biomarkers (Emdin et al., 2009).

Among these, human BNP is synthesized as a 134-amino acid precursor (pre-proBNP), which undergoes processing to form a 108-amino acid pro-peptide (proBNP). ProBNP is enzymatically cleaved by pro-protein convertases, such as corin and furin, into a 76-amino acid N-terminal fragment (NT-proBNP) and a 32-amino acid biologically active C-terminal fragment (BNP). Both NT-proBNP and BNP are released into circulation. Notably, O-glycosylation of proBNP within the Golgi apparatus can inhibit cleavage by corin and furin. The biologically active BNP1–32 binds to receptors NPR-A and NPR-C, with NPR-A mediating its effects and NPR-C functioning primarily for clearance (Semenov et al., 2010).

The clinical relevance of biochemical biomarkers in heart failure

Heart failure is defined as a syndrome resulting from structural or functional cardiac disorders that compromise the heart's ability to maintain physiological circulation. Diagnosing HF is not based on a single test. While a positive history and certain physical signs (e.g., orthopnea, rales, third heart sound, or jugular vein distension) offer good specificity, their sensitivity in diagnosing acute congestive HF is limited. Therefore, diagnosis relies on clinical judgment combining history, physical examination, and appropriate investigations, as per international guidelines (Thygesen et al., 2012). These guidelines strongly recommend natriuretic peptides, particularly BNP and NT-proBNP, as first-line biomarkers for diagnosing both acute and chronic HF, with the highest recommendation class (I) and evidence level (A). BNP and NT-proBNP testing are especially valuable in cases of unclear dyspnea etiology. Since the early 2000s, guidelines have established that lower BNP or NT-proBNP levels effectively exclude HF, while higher levels have a strong positive predictive value for HF diagnosis (Yancy et al., 2013).

From a pathophysiological perspective, the cardiac endocrine system is integral to maintaining fluid, electrolyte, and hemodynamic homeostasis, interacting closely with nervous and immunological systems and other organs such as the kidneys, endocrine glands, liver, adipose tissue, and immunocompetent cells. This interplay helps explain why BNP and NT-proBNP levels may increase in some non-cardiac conditions (Clerico et al., 2011).

Follow-Up and Risk Stratification in HF Patients

The levels of BNP and NT-proBNP serve as reliable indicators of the effectiveness of treatment in both acute and chronic heart failure (HF). A decline in these biomarkers over time is linked to improved clinical outcomes (Troughton et al., 2014). While most studies investigating biomarker-guided HF management strategies are small and lack sufficient power, three recent meta-analyses collectively confirm that BNP-guided therapy significantly reduces all-cause mortality and cardiovascular hospitalizations in HF patients compared to standard clinical care. This effect is especially pronounced in patients under 75 years of age with comorbidities (Januzzi & Troughton, 2013).

Regarding cardiovascular risk stratification in HF patients, the most recent guidelines strongly recommend the measurement of natriuretic peptides for prognostic purposes, supported by the highest level of evidence (class I, level A) [19]. Elevated levels of cardiac troponin I (cTnI) and troponin T (cTnT)—particularly when assessed using high-sensitivity methods—are frequently observed in HF patients, even in the absence of apparent myocardial ischemia or coronary artery disease (Januzzi et al., 2012). The presence of elevated cardiac troponin levels in chronic or acutely decompensated HF is associated with worse clinical outcomes and higher mortality rates. Patients who experience a substantial and sustained reduction in troponin levels following effective pharmacological treatment tend to have better prognoses than those with only transient or no reduction in these levels. Consequently, the latest guidelines recommend routine measurement of troponin I or T, alongside natriuretic peptides,

in patients with acutely decompensated HF for risk stratification, supported by the highest level of evidence (class I, level A).

Additional Biomarkers in HF Prognostication

Beyond natriuretic peptides and troponins, numerous other biomarkers have been suggested for prognostic purposes in HF, including those associated with pro-inflammatory mechanisms, oxidative stress, cachexia, neurohormonal dysfunction, and myocardial remodeling. For instance, biomarkers of myocardial fibrosis, such as galectin-3 (Van Der Velde et al., 2013) and soluble ST2 (Gruson et al., 2014), have been shown to predict hospitalization and mortality in HF patients. Accordingly, recent guidelines also suggest incorporating these biomarkers into risk stratification strategies, albeit with a lower degree of evidence compared to natriuretic peptides and troponins. For ambulatory HF patients, this recommendation is classified as IIb, level B, while for acute HF patients, it is classified as IIb, level A. Tables 3 and 4 summarize the pathophysiological and methodological characteristics of currently available biomarkers recommended by international guidelines based on evidence-based medicine principles.

Multi-Marker Approach to Cardiovascular Risk Evaluation

Emerging evidence suggests that future HF therapy may benefit from strategies combining multiple biomarkers—a methodology known as the multi-marker (MM) approach or global risk model. This approach is currently considered the most effective model for individual cardiovascular risk prediction. However, developing an adequate MM model is complicated by theoretical and methodological challenges. According to the MM approach, each biomarker should contribute independently to diagnostic and prognostic accuracy in a multivariable regression model, ultimately improving patient outcomes.

In 2010, the American Heart Association outlined criteria for evaluating novel cardiovascular risk markers, emphasizing the need for robust research design, representative at-risk populations, and sufficient outcome events. Studies should quantify how a novel marker adds prognostic value to established risk markers using measures of discrimination and accuracy. Furthermore, the clinical utility of a marker should be determined by its influence on patient management and outcomes (Hlatky et al., 2009). The evaluation of novel risk markers follows Evidence-Based Laboratory Medicine principles, progressing through phases such as proof of concept, prospective validation, documentation of incremental value, and cost-effectiveness assessment. Biomarkers that fail to impact disease management are unlikely to significantly influence patient outcomes or demonstrate cost-effectiveness in terms of quality-adjusted life-years gained. Randomized trials are the gold standard for validating biomarker-guided strategies, but such trials are scarce in cardiology, particularly in the context of primary prevention. This scarcity contributes to the relatively low evidence level (class IIa, level B) supporting BNP-guided therapy for chronic HF, as noted in recent guidelines. However, ongoing pivotal randomized clinical trials aim to clarify the utility of BNP-guided therapy in HF management (Januzzi & Troughton, 2013).

Challenges in Cardiac Biomarker Testing

Cardiovascular biomarkers are typically measured using non-competitive immunometric assays involving specific antibodies targeting separate epitopes of the biomarkers. Developing reliable and robust measurements for cardiovascular biomarkers poses significant challenges for laboratory medicine. Common cardiovascular biomarkers, such as natriuretic peptides, cardiac troponins, galectin-3, and ST2, are present in healthy individuals at extremely low concentrations (ng/L range). Consequently, immunoassays with high analytical sensitivity (detection limits of approximately 1 pg/tube or lower) are essential to achieve acceptable precision, particularly in pediatric populations (Apple & Collinson, 2012).

Certain biomarkers, such as BNP, consist of related peptide families, while others, like troponins, exhibit chemical and structural heterogeneity in circulation. This heterogeneity can lead to variable antibody interactions in immunoassays, potentially affecting measurement accuracy. As a result, significant differences exist between biomarker levels measured using different immunoassay methods. For example, Wu et al. [92] demonstrated in 1998 that commercial immunoassays yielded inconsistent results for identical protein concentrations due to complex forms of cTnI. Apple further highlighted the non-comparability of absolute concentrations across assays from different manufacturers. Commercial cTnI assays employ various standard materials and antibodies with distinct epitope specificities, resulting in unique outcomes for each assay and platform. Consequently, reference values and decision limits must be established for individual methods rather than extrapolated across assays, creating confusion in clinical practice, particularly for patients referred to multiple laboratories using different methods.

To address this issue, a study group representing international organizations such as AACC and IFCC initiated a standardization process for cTnI immunoassays in 2001. This effort aims to establish reference measurement procedures and materials to enhance comparability and reliability.

5. Standardization or Harmonization

The standardization of peptide and protein immunoassays, such as cTnI methods, remains a highly intricate undertaking. A comprehensive standardization framework requires the establishment of recognized reference measurement procedures (RMPs) and reference materials specific to cTnI, which are currently unavailable. Indeed, the term "standardization" can only be applied when comparable results across measurement procedures are achieved through calibration traceability to SI units utilizing RMPs.

Despite over a decade of efforts, some researchers argue that the standardization of cTnI assays is unlikely to be realized (Apple, 2012). Fred Apple advises laboratorians and clinicians to avoid becoming "bogged down" with the issue of cTnI standardization. Instead, he suggests focusing on comprehending the clinical and analytical evidence underlying cTnI immunoassays, advocating satisfaction with technological advancements that have facilitated precise detection of low cTnI concentrations, thereby enhancing patient care.

While we concur with Apple that standardizing certain critical immunoassay methods appears to be a formidable challenge akin to a "mission impossible", we nonetheless believe that achieving improved harmonization—defined as a reduction in heterogeneity among results from different methods—is attainable. Specifically, we propose that gaining deeper insights into the biochemical properties and pathophysiological roles of candidate biomarkers could drive harmonization by guiding manufacturers and laboratorians toward specific targets (e.g., epitopes) for the development of more accurate immunoassay methods. An illustrative example of potential harmonization can be observed in immunoassay methods for the cardiac B-type-related natriuretic peptide system.

6. The B-Type Cardiac Natriuretic Peptide System

The human BNP gene encodes a pre-proBNP molecule consisting of 134 amino acid residues, including a 26-amino-acid signal peptide. The prohormone molecule, proBNP1–108 (proBNP), contains 108 amino acids and is cleaved into two peptides prior to secretion: the biologically inactive NH2-terminal fragment proBNP1–76 (NT-proBNP) and the COOH-terminal peptide fragment proBNP77–108. The latter, a 32-amino-acid peptide (BNP1–32), is commonly referred to as BNP and represents the active hormone capable of binding to specific natriuretic peptide receptors (NPR-A, NPR-B, NPR-C).

Emerging studies have revealed a more intricate understanding of the pathophysiological and clinical significance of circulating B-type natriuretic peptides. Besides the active hormone BNP and the inactive NT-proBNP, numerous proBNP-derived fragments, including intact and glycosylated forms of proBNP, have been identified in human plasma through chromatographic techniques. Research indicates that intact or glycosylated forms of proBNP constitute a considerable portion of immunoreactive B-type peptides circulating in the plasma of heart failure (HF) patients (Miller et al., 2011).

Theoretically, the active hormone BNP may also be produced in vivo from circulating intact precursor proBNP through enzymatic cleavage by plasma proteases such as corin . A recent rat model study demonstrated that human proBNP can be processed into active BNP within the circulatory system. This peripheral processing could be regulated by mechanisms potentially impaired in HF patients, offering new therapeutic opportunities. A promising pharmacological approach might involve targeting drugs to induce or modulate the maturation of proBNP into BNP.

Methodologically, the considerable heterogeneity of B-type peptides in human plasma likely explains systematic discrepancies in BNP immunoassay results (Franzini et al., 2013). For instance, a recent study showed that certain methods, including Shionogi's IRMA and Siemens' ADVIA, yielded significantly lower BNP values compared to others like Alere's POCT Triage and Abbott's ARCHITECT methods. These differences have been partly attributed to cross-reactivity with glycosylated and non-glycosylated forms of proBNP.

7. What B-Type-Related Peptide Should We Measure and Why?

Given the evolving understanding of circulating B-type natriuretic peptides, there are at least three measurable forms in plasma: the active hormone BNP, the inactive NT-proBNP, and the precursor proBNP. Each peptide differs in biochemical properties and pathophysiological relevance (Table 6). Analytically, NT-proBNP and proBNP are more stable in vivo and in vitro, possessing longer plasma half-lives and lower intra-individual variability than BNP. Pathophysiologically, studies suggest that inactive peptides, particularly proBNP, demonstrate stronger associations with HF progression than BNP.

Notably, mass spectrometry studies have revealed that active BNP1–32 concentrations in severe HF patients are significantly lower than values reported by immunoassays. Furthermore, some studies detected no measurable active BNP1–32 in advanced HF patients, highlighting limitations in current assay methodologies.

Although NT-proBNP and proBNP appear to be better biomarkers for HF progression, commercial immunoassays for BNP often cross-react with these inactive peptides. Nevertheless, guidelines indicate that BNP and NT-proBNP immunoassays provide clinically comparable results for HF diagnosis, prognosis, and follow-up.

This creates a paradox: from a pathophysiological perspective, measuring active BNP would provide a more accurate assessment of cardiac endocrine function. However, currently available methods fail to achieve specificity for BNP1–32, being significantly influenced by inactive peptides in HF patients.

In conclusion, discrepancies in BNP immunoassay results arise from interference by inactive peptides, particularly glycosylated and non-glycosylated proBNP forms. Developing more specific assays for active BNP1–32 could improve harmonization. Future approaches may involve dual-method testing to measure proBNP and BNP concurrently, enhancing our understanding of cardiac endocrine dysfunction in HF. Rigorous evaluation of these new methods in clinical trials will be essential to determine their utility compared to existing NT-proBNP and proBNP assays, following evidence-based principles.

Troponin Molecule and Biology

The troponin complex, present in both striated skeletal and cardiac muscle tissues, consists of three distinct polypeptides: troponin C, troponin T, and troponin I. Troponin C functions as the calcium-binding subunit, troponin T as the tropomyosin-binding component, and troponin I regulates the actin-myosin interaction by inhibiting the activity of actomyosin adenosine triphosphatase. Collectively, the troponin complex regulates muscle contraction by associating with thin filaments within the sarcomere.

Multiple isoforms of troponin T and I have been identified, with differential expression patterns in fetal and adult cardiomyocytes and skeletal muscle cells. Adult cardiomyocytes express only one isoform, while skeletal muscle cells downregulate this isoform in favor of skeletal muscle-specific variants. Consequently, troponin T and I in adult cardiac and skeletal muscle tissues are both genetically and antigenically distinct. Conversely, troponin C found in skeletal muscle is identical to its cardiac counterpart. The isoforms of troponin I and T found in adult cardiac tissues are therefore referred to as cardiac troponin I and cardiac troponin T, respectively.

The molecular weights of cardiac troponin I and T differ, with cardiac troponin T weighing approximately 38 kDa and cardiac troponin I approximately 24 kDa. These cardiac troponins are distributed in two distinct pools within cardiomyocytes. Over 90% of cardiac troponin I and T are bound to the myofibrillar structure, whereas a smaller fraction exists unbound in the cytosol. Troponins from the free cytosolic pool are released relatively quickly, typically within one to two hours following myocardial injury. Clinical observations reveal that transient elevations in circulating cardiac troponins can occur in the absence of overt myocardial necrosis, such as during episodes of paroxysmal supraventricular tachycardia. In contrast, the troponins bound to the myofibrillar apparatus are released more slowly and persistently following cell necrosis, allowing detection in the bloodstream over several days after the initial injury.

The mechanisms involved in the clearance of cardiac troponins are not fully understood, though their circulating half-lives are estimated to be approximately two hours [6]. Cardiac troponins may undergo various structural modifications during circulation, including proteolysis, phosphorylation, and oxidation. Renal failure has been associated with elevated circulating cardiac troponin levels, a phenomenon often attributed to impaired renal clearance. However, evidence suggests that enhanced release mechanisms may play an equally significant role. For example, renal transplantation in end-stage renal failure patients does not consistently reduce circulating cardiac troponin levels.

Beyond renal failure, several factors have been identified as influencing circulating troponin concentrations, including age, gender, and comorbidities such as diabetes mellitus, hypertension, and atherosclerotic disease. These variables may hold clinical significance when interpreting troponin levels in patients suspected of acute heart failure (HF) or acute coronary syndrome (ACS).

The widespread adoption of biomarker-guided therapy in HF and ACS raises important considerations regarding cost-effectiveness and resource allocation. While these tests provide critical insights into disease management, their financial burden on healthcare systems must be evaluated. Comparative studies assessing the clinical outcomes and cost implications of single-marker versus multi-marker strategies could guide policymakers in optimizing resource utilization. Additionally, efforts to standardize and streamline biomarker assays could further reduce costs while improving diagnostic consistency.

Future Perspectives in Laboratory Diagnosis

The integration of artificial intelligence (AI) and machine learning (ML) into biomarker-based diagnostics offers promising avenues for enhancing diagnostic precision and prognostic modeling. Algorithms trained on large datasets of biomarker profiles and clinical outcomes could identify patterns and predict disease trajectories more accurately than traditional methods. Such technologies have the potential to facilitate earlier detection of HF and ACS, allowing for timely therapeutic interventions. However, the ethical considerations, data standardization, and cross-platform interoperability of AI-based diagnostic tools must be addressed to ensure their widespread applicability.

Conclusion

This paper emphasizes the critical role of biomarkers, particularly cardiac troponins and natriuretic peptides, in the diagnosis, prognosis, and management of heart failure (HF) and acute coronary syndromes (ACS). The evolution of laboratory diagnostic techniques has been pivotal in shaping our understanding of these biomarkers, as well as their underlying pathophysiology. Despite substantial progress, the lack of standardization in immunoassays exemplified by the variability in results for cardiac troponins and BNP highlights an ongoing challenge in clinical practice. Harmonization of assay methodologies and the development of specific immunoassays could greatly enhance diagnostic accuracy and reliability.

Further, the paper explores the biology of cardiac troponins and natriuretic peptides, their unique characteristics, and their implications for cardiovascular risk stratification. By integrating traditional single-marker approaches with emerging multi-marker strategies, future diagnostic models may provide a more comprehensive assessment of cardiac function and disease progression. This advancement is expected to not only improve patient outcomes but also inform personalized therapeutic interventions. However, these methodologies must be validated through rigorous clinical trials to ensure their efficacy and cost-effectiveness.

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