

BNP and NT-proBNP: A Review of Analytical Aspects and Clinical Utility

BNP et NT-proBNP : Revue des aspects analytiques et de l'utilité clinique

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Keywords: Cardiac biomarkers, Heart failure, Clinical decision-making, Laboratory diagnostics, Healthcare utilization

Abstract

This article provides an update on the current knowledge regarding natriuretic peptides, particularly BNP and NT-proBNP, which have become essential biomarkers in the management of heart failure. Since their discovery, these markers have established themselves as indispensable non-invasive tools for monitoring this condition. We discuss their structural, metabolic, and analytical aspects, along with clinical recommendations for their use. Understanding the factors that influence their levels is fundamental for accurate interpretation in daily practice. This review aims to provide biologists and clinicians with the necessary information to optimize the use of these biomarkers in the monitoring of heart failure patients.

Résumé

Cet article fait le point sur les connaissances actuelles relatives aux peptides natriurétiques, en particulier le BNP et le NT-proBNP, devenus des biomarqueurs essentiels dans la prise en charge de l'insuffisance cardiaque. Depuis leur découverte, ces marqueurs se sont imposés comme des outils de référence non invasifs pour le suivi de cette pathologie. Nous abordons leurs aspects structurels, métaboliques et analytiques, ainsi que les recommandations cliniques pour leur utilisation. Comprendre les facteurs influençant leurs concentrations est fondamental pour une interprétation précise dans la pratique quotidienne. Cette mise au point vise à offrir aux biologistes et cliniciens les informations nécessaires pour optimiser l'utilisation de ces biomarqueurs dans le suivi des patients cardiaques.

Introduction:

In the latest recommendations from cardiology societies, notably the Guidelines of the European Society of Cardiology (ESC) published in 2021 [1, 2], and those of the American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA) published in 2022 [3], only natriuretic peptides (NPs) are cited as useful markers for the management of patients suffering from heart failure (HF). Their measurement is recommended for screening, exclusion diagnosis, risk stratification, prognosis, and monitoring of these patients.

Natriuretic peptides are a family of hormones comprising several members: ANP (Atrial Natriuretic Peptide), composed of 28 amino acids (AA), secreted by the atrium; BNP (Brain Natriuretic Peptide), composed of 32 AAs, secreted by cardiomyocytes; CNP (C-type Natriuretic Peptide), composed of 22 AAs, originating from endothelial cells; DNP (Dendroaspis Natriuretic Peptide), isolated from the venom of a mamba snake; VNP (Vasopeptide or Type V Natriuretic Peptide); and urodilatin, which originates in the kidneys [4]. All these NPs share a similar structure characterized by a 17-AA ring formed by a disulfide bond [5].

There is significant homology between the structures of ANP and BNP, which are primarily synthesized by cardiomyocytes. ANP, the first identified member of the natriuretic peptide family, was isolated and purified in 1984 [6]. BNP was initially isolated from the porcine brain in 1988 (hence its original name “Brain Natriuretic Peptide”), and it was detected in the human heart in 1991 [7].

For the diagnosis and monitoring of HF, only BNP, N-terminal proBNP (NT-proBNP), and the mid-regional pro-atrial natriuretic peptide (MR-proANP), a central fragment of the ANP precursor, are currently recommended by cardiology societies [8, 9].

However, the effective use of these biomarkers in clinical practice relies on a thorough understanding of their structural, metabolic, and analytical characteristics, as well as current clinical guidelines.

This review revisits the current knowledge on natriuretic peptides, focusing on BNP and NT-proBNP. It clarifies the structural, metabolic, and analytical aspects of these biomarkers and their practical applications, while presenting recent clinical recommendations for their use. The goal is to provide biologists and clinicians with the necessary information to optimize the use of these biomarkers in the monitoring of cardiac patients.

Synthesis of cardiac natriuretic peptides

ANP and BNP are secreted in response to various stimuli, with the primary trigger being cardiomyocyte stretching. They are synthesized as preprohormones. ANP is produced as a preprohormone of 151 amino acids (AA), which undergoes intracellular proteolysis to form the 126-AA proANP. This proANP is then stored in granules of atrial myocytes. Upon secretion, it is cleaved into active ANP (28 AA) and NT-proANP (98 AA). Under physiological conditions, this peptide is mainly synthesized in the atrium. However, in cases of heart failure, it can also be produced by the left ventricle.

BNP, on the other hand, is synthesized as a preprohormone of 134 amino acids. It is cleaved into a signal peptide and 108-AA proBNP. The latter undergoes further proteolysis upon secretion, mediated by furin and/or corin, yielding active BNP (32 AA) and inactive NT-proBNP (76 AA). Unlike ANP, BNP is not stored; it is secreted immediately after synthesis.

In addition to myocardial fiber stretching, other stimuli for BNP secretion have been reported, including interleukin-1 (IL-1), tumor necrosis factor α (TNF α), transforming growth factor β (TGF β), lipopolysaccharides, and cellular hypoxia [5]. Like ANP, BNP is primarily synthesized in the atrium. However, in heart failure, its production by the left ventricle surpasses that of ANP.

This article will focus on BNP and NT-proBNP, which are the most studied and measured in the context of heart failure.

Physiological effects of BNP

Natriuretic peptides exert their effects through membrane receptors, Natriuretic Peptide Receptors A and B (NPR-A and NPR-B), which activate guanylyl cyclase and produce cyclic GMP [10]. BNP has multiple effects (Figure 1) :

- **Cardiovascular system:** BNP reduces blood pressure by inducing vasodilation and increasing vascular permeability. These effects are enhanced by reducing sympathetic tone [11].
- **Renal system:** BNP counteracts volume expansion by increasing diuresis and natriuresis, through enhanced glomerular filtration and effects on renal tubules, inhibiting the activity of the Na^+/H^+ exchanger in the proximal tubule and the Na^+/Cl^- cotransporter in the distal tubule [12, 13]. It also inhibits renin and aldosterone synthesis [14].
- **Central nervous system:** BNP reduces salt appetite and diminishes thirst [13].
- **Cardiac remodeling:** BNP has anti-remodeling properties, reducing fibrosis and hypertrophy induced by growth factors [5].

A third receptor type, the Natriuretic Peptide Receptor C (NPR-C), mediates the intracellular clearance of NPs [4].

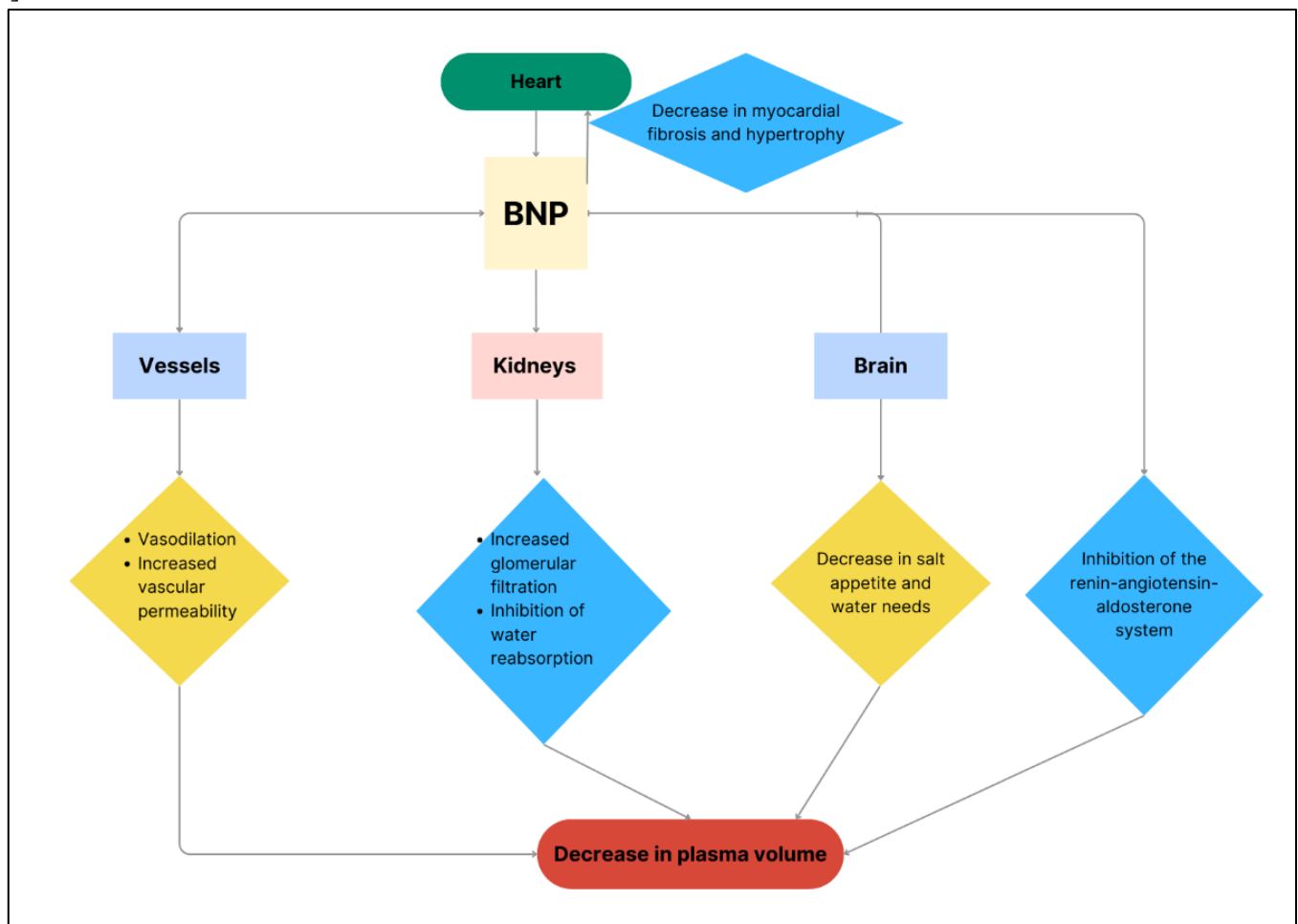


Figure 1. Physiological effects of BNP.

Clearance

The active form of BNP is secreted in equimolar amounts with NT-proBNP (inactive). Additionally, proBNP (inactive) is also present in plasma (Figure 2). Clearance of the active form primarily occurs in the vasculature via endocytosis mediated by NPR-C. The active forms are proteolyzed by a membrane-bound endothelial cell metalloprotease, which contains a zinc atom at its active site—neutral endopeptidase 2.4.11 (NEP) or neprilysin.

Other enzymes involved in BNP 1-32 (active form) degradation include dipeptidyl peptidase IV (DPP IV), a surface protease also found in soluble plasma form, which cleaves BNP 1-32 into BNP 3-32 [15]. Insulin-degrading enzyme (IDE) and meprin are additional enzymes involved in BNP degradation [16]. Notably, degraded forms of BNP 1-32 also retain *in vivo* activity, albeit reduced compared to intact BNP 1-32 [16, 17]. The enzymes that cleave BNP 1-32 are also capable of cleaving proBNP and NT-proBNP [16], although NT-proBNP clearance is predominantly renal.

The plasma half-life of BNP is approximately 20 minutes, while that of ANP is only a few minutes. In contrast, NT-proBNP has a half-life of up to 120 minutes.

Circulating forms

The activity of various enzymes involved in BNP metabolism gives rise to multiple circulating forms in the blood (Figure 2): intact forms of ProBNP, BNP, NT-proBNP, and the signal peptide; glycosylated forms of ProBNP and NT-proBNP [16] (BNP is not glycosylated [18]); and truncated forms of ProBNP, BNP, and NT-proBNP [19]. It should be noted that ProBNP (glycosylated and non-glycosylated) constitutes the predominant circulating form [18, 20].

The presence of these various forms may explain the difficulty in assay standardization and result interpretation. The available assay methods are immunologically based, using antibodies that recognize these different circulating forms. A lack of rigorous standardization in these methods may lead to discrepancies in results across commercial manufacturers, with implications for interpreting values against thresholds set by professional societies and the resulting therapeutic decisions.

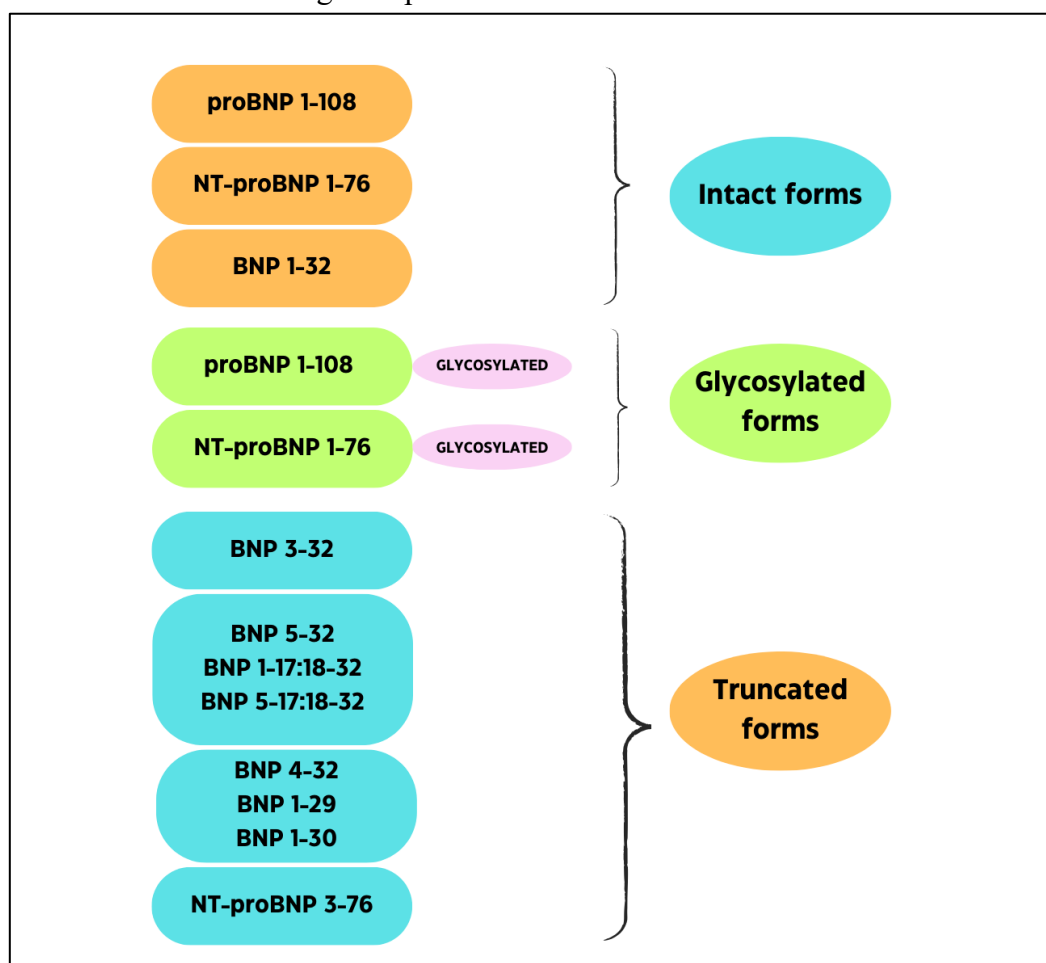


Figure 2. Complexity of the circulating molecular forms of B-type natriuretic peptide.

Assay methods

NPs assays employ immunological techniques that differ in the nature of the measured product (active form or N-terminal product), the antibodies used, the calibrator, and the detection type. The intact form of ANP is not commonly assayed because its diagnostic performance is inferior to that of BNP and NT-proBNP. However, the measurement of Mid-regional pro-atrial natriuretic peptide (MR-proANP) appears to offer a distinct alternative to BNP and NT-proBNP for diagnosing and monitoring heart failure [21], although this new biomarker is not yet routinely used in clinical practice.

The first commercial methods for BNP assay utilized isotopic detection with iodine-125, which were unsuitable for emergency use. Subsequently, a fluorometric point-of-care test (POCT) was developed for whole blood, enabling bedside use. Biosite's Triage kit, the first of its kind, was launched at the end of 2000. Since then, simpler methods, such as sandwich assays with chemiluminescence or fluorimetry detection, have been developed on various automated platforms, with analysis times suitable for emergency use (Table 1).

Table 1 Example of commercially available automata for NPs dosing.

PNs	Automate	Firm
BNP	Architect, AxSYM, iSTAT	Abbott
	Triage	Alere
	Access, Access 2, DxI	Beckman Coulter
	Advia Centaur, VISTA Dimension, ExL dimension	Siemens healthcare
	Tosoh AIA	Tosoh Bioscience
NT-proBNP	Triage	Alere
	VIDAS	bioMerieux
	Vitros ECI	Ortho Clinical Diagnostics
	AQT90 FLEX	Radiometer
	RAMP	Response Biomedical
	Elecsys, cobas and cobas h 232 POC system	Roche Diagnostics
	RxL dimension, Stratus CS, VISTA dimension, Dimension EXL, Immulite	Siemens healthcare
MR-proANP	KRYPTOR	Thermo Fisher Scientific

These commercial methods use different reagents, including monoclonal antibodies targeting specific epitopes: the C-terminal region (residues 27–32), the loop sequence (14–21), or the disulfide bond [22], which explains the variability in results obtained by different analyzers. Additionally, BNP assays recognize recombinant BNP ("Nesiritide"), a drug used in some countries to treat heart failure, complicating result interpretation.

For NT-proBNP, commercial methods utilize the same antibodies and calibrators, resulting in better standardization [23]. BNP, a fragile molecule, requires blood collection in plastic tubes with EDTA. Contact with glass can activate certain coagulation proteins, leading to BNP degradation. Prompt centrifugation and plasma separation are imperative, with plasma needing freezing for storage beyond 24 hours. NT-proBNP, being more stable, can be measured in serum or plasma treated with heparin or EDTA. After centrifugation, it remains stable for 72 hours at room temperature, at least six days at 4°C, and one year at -20°C [5, 14] (Table 2).

Table 2 Main differences between BNP and NT-proBNP.

	BNP	NT-proBNP
Properties		
Number of amino acids	32	76
Molecular weight	3.5 kD	8.5 kD
Hormonal activity	Yes	No
Plasma half-life	20 min	60-120 min
Disposal mode	Cleavage by an endopeptidase, internalization by clearance receptor	Mainly renal
Pre-analytical		
Type of collection	Plasma (EDTA)	Serum or plasma (Heparin or EDTA)
Conservation		
Ambient temperature	4 to 24 h (whole blood or EDTA plasma)	72 h (after centrifugation)
+4°C	24 to 48 hours	6 days
-20°C	Not available	1 year
Analytical		
Standardization	-	+/-
Interference with Nesiritide	+	-
Interference with ProBNP	+/-	+/-
POCT (point-of-care testing)	+	+

Regardless of the circumstances, adherence to manufacturers' recommendations is essential. Inter-method variations, though moderate, can influence result interpretation. Therefore, it is crucial to monitor the same patient using the same method in the same laboratory. A strong correlation exists between BNP and NT-proBNP values, with their diagnostic and prognostic performance being closely aligned. The choice between these tests depends solely on laboratory equipment availability, though their values are not interchangeable.

Physiological variations

Several factors influence BNP and NT-proBNP plasma levels, necessitating consideration for accurate interpretation. BNP and NT-proBNP levels are elevated on the first day of life, gradually declining until the third month, and stabilizing until puberty [24, 25]. In adults, their concentrations increase with age, with a more pronounced rise after 75 years [26, 27]. Women exhibit higher plasma levels of BNP and NT-proBNP compared to men [27].

Plasma levels of these peptides are inversely correlated with body mass index (BMI) [28], possibly due to high NPR-C receptor expression in adipose tissue [29]. Consequently, decision thresholds should be lowered for obese individuals and raised for lean individuals relative to those with normal BMI.

Renal function also affects NPs plasma levels; BNP increases are modest in renal failure patients. Since renal function deteriorates systematically with age, a decision threshold adjustment to 200 ng/L (instead of 125 ng/L) has been proposed when clearance drops below 60 mL/min/1.73 m² [5]. NT-proBNP, primarily cleared renally, complicates interpretation in patients with renal insufficiency.

Finally, due to the pulsatile secretion of NPs, BNP and NT-proBNP levels may vary throughout the day, resulting in significant intra-individual variations of 30–50% in both healthy and stable heart failure patients. Significant interpretation of NPs results requires variations exceeding 50% [30].

Decision thresholds

Given the challenges of establishing unified reference values, several decision thresholds have been proposed since 2005, either age-dependent or context-dependent (acute or chronic). The European Society of Cardiology (ESC) has defined NPs thresholds for ruling out acute cardiac-origin dyspnea, differing by acute or non-acute context (Table 3) [1, 8].

Table 3. Exclusion thresholds for dyspnea of cardiac origin according to the European Society of Cardiology.

Natriuretic peptide	Non-acute context	Acute context
BNP	35 pg/mL	100 pg/mL
NT- proBNP	125 pg/mL	300 pg/mL
MR- proANP	40 pmol/L	120 pmol/L

For preserved ejection fraction heart failure (HFpEF) diagnosis, the BNP and NT-proBNP thresholds are the same for patients in sinus rhythm. For atrial fibrillation (AF) patients, the thresholds are: BNP $\geq$ 105 pg/mL, NT-proBNP $\geq$ 365 pg/mL [1].

Pathological variations

Elevated NPs plasma levels result from enhanced synthesis, impaired clearance, or both.

Natriuretic peptides and heart failure

It is well-established that NPs plasma levels are elevated in HF. Their measurement is recommended for:

Diagnostic aid: Since 2005, NPs measurement has been systematically recommended by the ESC for diagnosing acute and chronic HF [31]. The ESC advocates measuring BNP, NT-proBNP, or MR-proANP to rule out HF due to their high negative predictive value [1, 8, 32]. NPs use is recommended for exclusion, not HF diagnosis establishment [1], as their positive predictive values are insufficient, risking misdiagnosis. The ESC diagnostic algorithm integrates clinical examination, risk factors, ECG, NPs measurement, and echocardiography (ECHO) [1]. ECHO remains the reference test to confirm HF, though it may not always be available in emergency settings. NPs measurement, being readily accessible, is invaluable diagnostically. If BNP or NT-proBNP levels are below the threshold, ECHO is unnecessary.

Screening: Although echocardiography detects early ventricular dysfunction, it is impractical for mass screening. The utility of BNP and NT-proBNP has been evaluated for screening systolic and/or diastolic ventricular dysfunction. NPs levels correlate with ventricular dysfunction severity, including preserved systolic function [33]. However, their value in this context is modest, except in specific populations (relatively young, without significant comorbidities) where discriminatory values are low. Natriuretic peptides are thus more effective in detecting subclinical cardiac disorders than assessing precise degrees of ventricular dysfunction.

Prognosis

BNP and NT-proBNP are also independent prognostic markers of morbidity (rehospitalization) and mortality [34, 35]. It is recommended to measure these biomarkers at admission and discharge for prognostic purposes in the management of heart failure.

Therapeutic monitoring

The excellent predictive value of natriuretic peptides and the reduction in their concentrations under validated treatments for chronic heart failure have led to several studies [36-46] aimed at evaluating their usefulness in guiding patient management and adjusting treatment. Unfortunately, the results are inconclusive and sometimes contradictory.

At present, there is insufficient data to make firm recommendations on target NPs levels or the levels at which treatment should be adjusted. However, increasing NPs concentrations are linked to worsening heart failure, particularly in patients under 75 years of age with reduced ejection fraction. It remains unclear how often natriuretic peptides should be measured and whether it is better to target a specific NPs level or reduce NPs levels by a specific percentage.

It is important to note that when a patient is treated with an ARNi (angiotensin receptor neprilysin inhibitor), which combines an angiotensin receptor antagonist and a neprilysin inhibitor, measured BNP concentrations are elevated. NT-proBNP concentrations do not appear to be affected by this treatment. Some authors suggest using only NT-proBNP as a cardiac biomarker for monitoring patients on ARNi therapy [47].

Natriuretic peptides and other pathologies

In addition to heart failure, numerous cardiac and non-cardiac conditions can lead to elevated plasma levels of BNP and NT-proBNP, which must be considered when interpreting NPs levels (Table 4).

Table 4 Diseases that can lead to increased plasma levels of BNP and NT-proBNP.

Cardiac origin	Non-cardiac origin
- Myocardial infarction (MI)/Acute coronary syndrome (ACS)	- Chemotherapy
- Atrial fibrillation (AF)/atrial flutter	- Pulmonary hypertension
- Myocarditis / cardiomyopathies	- Diabetes mellitus
- Cardiac hypertrophy	- Chronic obstructive pulmonary disease
- Valvulopathies and pericardial diseases	- Anemia
- Cardiac surgery and cardioversion	- Renal failure
	- Bacterial sepsis
	- Extensive burns

Conclusion

Over the past decade, numerous biomarkers reflecting different aspects of the molecular interactions involved in heart failure have emerged and seem to hold potential in the management of this condition. Currently, only natriuretic peptides remain recommended by scientific societies. These markers have proven their utility and are an essential tool in managing patients with heart failure. Proper use of these markers depends on the clinician's and biologist's understanding of the analytical and pre-analytical aspects, as well as the factors that may influence their plasma concentrations.

Disclosures

The author declares that there is no conflict of interest.

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